# Recovery of vegetation and bees after removal of pine forests by fire in the Limietberg region of the Cape Floristic Region biodiversity hotspot

By

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Thesis presented in partial fulfilment of the requirement for the degree of Master of Science at the University of Stellenbosch

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October 2015

**Declaration**

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

**Muhammed Nizaar Gardee**
Date: 27 October 2015

**General Abstract**

The present global biodiversity crisis is characterized mostly by loss of species due to habitat destruction but there other major threats – notably invasive alien species. The term "biodiversity hotspot" has been coined to emphasize areas for conservation prioritization – areas that have high biodiversity under threat from habitat destruction, invasive species, etc. The Cape Floristic Region (CFR) in the Western Cape (WC) Province of South Africa has the highest biodiversity of all Mediterranean-type climate regions globally and is classified among global biodiversity hotspots. The CFR, a Mediterranean-type climate heathland with a naturally high fire frequency, faces threat from transformation by development, disturbed (increased) fire frequencies and intensities, and invasive organisms, most notably invasive trees. Such threats disturb keystone species and keystone processes including insect pollinator assemblages and associated insect flower visitation webs and frequencies.

Invasive pines are a serious threat to insect flower visitation as pine trees (*Pinus* spp.) shade out much indigenous vegetation in the CFR. Little is known of their long-term effects on insect flower visitors and vegetation recovery in post-pine restoration and recovery areas after such trees are removed. I investigated the recovery of vegetation and the most important insect pollinator, bees, after the removal of pines by fire and passive recovery in a CFR valley in the Western Cape.

In 1999, a wildfire burned much of the WC Limietberg Nature Reserve along with an adjacent pine tree forestry stand - which was then left to recover, providing an ideal opportunity to investigate the enduring effects of pine afforestation in the CFR. In two data chapters, I compared the post-fire passive recovery of vegetation (Chapter 2) and bee diversity (Chapter 4) in areas which had previously been planted with pines vs. those which had contained natural fynbos. To improve on sampling methodology, I conducted an experiment that demonstrated the Observer Effect in bee sampling with a sweep net (Chapter 3), and I developed a novel sampling device (Chapter 5) for insect flower visitors.

Sampling of vegetation and bee diversity was conducted in a paired sampling design, where fynbos (Natural) sub-sites were paired with sub-sites which had previously been afforested with pines (Post-Pine Recovery; PPR) and the two sub-sites were separated by a distinct, linear boundary (Natural/PPR boundary). Sampling was conducted along transects parallel to the boundary and extending in both directions from the boundary into the Natural and in the opposite direction into PPR sub-sites. Five transects were positioned at 3, 10, 20, 30, and 40 m from the boundary (Ecotone) and three were positioned at 60, 80, and 100 m from the boundary (Deep).

In Chapter 2, I found that natural sub-sites consistently had higher total plant species abundance and species richness than PPR sub-sites. Approximately two thirds of plant species were more abundant in Natural sub-sites than in PPR sub-sites. There was no significant correlation in species richness or abundance with distance from the Natural/PPR boundary. Some genera are cautiously indicated as having

lower success in recovery after pine afforestation: *Erica* spp., *Restio* spp., *Hypodiscus* spp. while *Helichrysum* spp. is also tentatively indicated to recover well in PPR sub-sites. Soil disturbance and concomitant disruption of normal ecosystem functions, including pollination, is indicated as a probable reason for disruption of plant recovery.

In Chapter 3, sweep netting methodology was tested for the Observer Effect. A noticeable increase in bee visitation frequency to a common generalist plant species in bloom, *Metalasia densa,* was correlated with longer waiting periods after I stopped moving indicating the presence of an Observer Effect. This suggests that sweep netting for bees should only be commenced after a waiting period of five minutes during which the sweep netter is motionless.

In Chapter 4, using a flight-intercept modified pan trap, I compared bee species richness and bee species abundance across different seasons and in both mass-flowering and sparser flower patches. There was no significant difference in bee species richness between Natural and PPR sub-sites. All bee species, except one complex, were more abundant in Natural sub-sites. Nearly two thirds of all bee species (n=37 of 56) caught with sweep netting and the modified pan trap are undescribed species. Similar to the vegetation, the effects of soil disturbance as a result of decades of pine forest shading and pine forest litterfall followed by an unusually hot pine forest fire are indicated as the most likely reasons for lower bee abundance in PPR sub-sites. This is due to the difficulty associated with viable nest establishment and suitable pollen and nectar forage availability in disturbed areas.

In Chapter 5, the newly developed Pan and Flight Intercept Combination (PAFIC) trap's design, pilot testing, and comparison with the traditional pan trap is discussed. A preliminary test suggests that the PAFIC trap is more efficient (with higher abundance) than the traditional pan trap.

In Chapter 6, I discuss the implications of the disturbance of pine forestry and unusual pine fire to plant species and bee species assemblages. Bee-pollination webs in PPR sub-sites are indicated as being substantially simpler than those in Natural areas as well as compositionally different. The recovery of pollination as a keystone process in post pine-afforestation areas faces a substantial challenge given the disturbance to soil that decades of pine afforestation followed by pine forest fire can cause. Some suggestions are made for the restoration of fynbos areas recovering from pine afforestation including a discussion of augmenting re-vegetation measures (fynbos seed dispersal and seedling planting) with methods of restoring of healthy pedogenesis, epigaeic arthropod communities, and fynbos seedbanks.

**Algemene Opsomming**

Die huidige globale biodiversiteit krisis is gekenmerk meestal deur die verlies van spesies as gevolg van die vernietiging van habitat. Maar daar is ook ander groot bedreigings – veral merkbaar indringerspesies. Die term “biodiversiteit brandpunt” is geskep om gebiede vir bewaring prioritisering te beklemtoon - gebiede wat 'n hoë biodiversiteit bevat wat bedreig word deur die vernietiging van habitat, indringerspesies, ens. Die Kaap Floristiese Streek (KFS) in die Wes-Kaap (WK) Provinsie van Suid-Afrika het die hoogste biodiversiteit van alle Mediterreense tipe klimaat streke wêreldwyd, en is onder die globale biodiversiteit brandpunte geklassifiseer. Die KFS, 'n Mediterreense-tipe klimaat heideveld met 'n natuurlike hoë brand frekwensie, word bedreig as gevolg van transformasie deur ontwikkeling, versteurde (verhoogde) vuur regimes, en indringer organismes, veral indringerbome. Sulke bedreigings versteur hoeksteen spesies en die hoeksteen prosesse, insluitende insek bestuiwer samestelling en verwante insek blom besoekings webbe en frekwensies.

Indringer dennebome (*Pinus* spp.) is 'n ernstige bedreiging vir insek blom besoeking want dennebome verskaf skaduwee aan baie inheemse plantegroei in die KFS. Min is bekend oor die langtermyn effekte op insek blom besoekers en plantegroei restorasie na denneboom verwydering en restorasie gebiede na sodanige bome verwyder word. Ek ondersoek die restorasie van plantegroei en die belangrikste insek bestuiwer, bye, na die vernietiging van dennebome deur vuur en passiewe herstel in 'n KFS vallei in die Wes-Kaap.

In 1999 het'n veldbrand baie van die WK Limietberg Natuurreservaat saam met 'n aangrensende denneboom bosbouplantasie gebrand - wat toe gelaat is om te herstel via intermediêre suksessie na inheemse fynbos plantegroei. Dit bied 'n ideale geleentheid om die blywende gevolge van denneboom aanplanting in die KFS te ondersoek. In twee data hoofstukke, vergelyk ek die passiewe herstel van plantegroei na ‘n brand (Hoofstuk 2) en by diversiteit (Hoofstuk 4) in gebiede wat voorheen geplant is met dennebome teenoor diegene wat natuurlike fynbos vervat het. Om te verbeter op steekproefmetodiek, het ek 'n eksperiment onderneem wat demonstreer hoe die Waarnemer Effek by monsterneming met 'n vee-net beïnvloed (Hoofstuk 3), en het ek 'n nuwe monsterneming toestel ontwerp (Hoofstuk 5) vir insek blom besoekers.

Monsterneming van plantegroei en by diversiteit is uitgevoer in 'n gegroepeerde steekproef ontwerp, waar fynbos (Natuurlik) sub-terreine gegroepeer is met sub-terreine wat voorheen aangeplant was met dennebome (Na Denneboom Verwydering Herstel; NDVH) en die twee sub-terreine is geskei deur 'n duidelike, lineêre grens (Natuurlik / NDVH grens). Steekproefneming is gedoen langs transekte parallel tot die grens en versprei in albei rigtings van die grens tot binne die natuurlike fynbos en in die teenoorgestelde rigting tot binne NDVH sub-terreine. Vyf transekte is geposisioneer binne 50 m van die grens (Oorgang sone) en drie is ver geplaas van die grens (Diep).

In Hoofstuk 2 het ek gevind dat natuurlike sub-terreine konsekwent hoër totale getal plant individue

en spesierykheid as NDVH sub-terreine gehad het. Ongeveer twee derdes van plantspesies was meer volop in natuurlike sub-terreine as in NDVH sub-terreine. Daar was geen beduidende korrelasie in spesierykheid of totale hoeveelheid plant individue met betrekking tot afstand van die Natuurlike /NDVH grens. Sommige genera word versigtig aangedui as met laer sukses in die restorasie terreine na denneboom aanplanting: *Erica* spp., Restio spp., *Hypodiscus* spp. terwyl *Helichrysum* spp. voorlopig aangedui is as goed herstel in NDVH sub-terreine. Grondversteuring en gepaardgaande ontwrigting van normale funksionering van die ekosisteem, insluitend bestuiwing, word aangedui as 'n waarskynlike rede vir die ontwrigting van die herstel van plantegroei.

In Hoofstuk 3 is vee-net metodologie getoets vir die Waarnemer Effek. 'N merkbare toename in by besoeking frekwensie van 'n algemene plantspesie in blomtyd, *Metalasia densa*, is gekorreleer met langer wagtydperke nadat ek gestop het om te beweeg, en dui dus op die teenwoordigheid van 'n Waarnemer Effek. Dit dui daarop dat die vee-net vir bye eers gebruik moet word na 'n wagtydperk waartydens die waarnemer bewegingloos verkeer.

In Hoofstuk 4, met behulp van 'n vlug-onderskep gewysigde pan lokval, het ek by spesierykheid en totale by hoeveelheid vergelyk oor verskillende seisoene en in beide massa-beblomde en yler blom kolle. Daar was geen beduidende verskil in by spesierykheid tussen natuurlike en NDVH sub-terreine. Alle by spesies, behalwe een spesie kompleks, was meer volop in natuurlike sub-terreine. Byna twee derdes van alle by spesies (n = 37 van 56) gevang met vee-nette en die van die gewysigde pan lokvalle is onbeskryfde spesies. Soortgelyk aan die plantegroei, is die gevolge van grondversteuring as gevolg van dekades van denneboom bos skadu en denneboom bos blaar afval gevolg deur 'n buitengewoon warm denneboom bos brand as die mees waarskynlike redes vir laer by hoeveelheid in NDVH sub-terreine aangedui. Dit is as gevolg van die probleme wat verband hou met lewensvatbare nes vestiging en geskikte stuifmeel en nektar voer beskikbaarheid in versteurde gebiede.

In Hoofstuk 5 is die nuut ontwikkelde Pan en Vlug Onderskep Kombinasie (PEVOK) lokval ontwerp, aanvanklike toetsing, en vergelyking met die tradisionele pan lokval bespreek. 'N Voorlopige toets dui daarop dat die PEVOK lokval meer doeltreffend (met 'n hoër oorvloed) is as die tradisionele pan lokval.

In Hoofstuk 6 bespreek ek die implikasies van die versteuring van denneboom bosbou en ongewone denneboom vuur met betrekking tot plant spesies en by spesies samestelling. By-bestuiwing webbe in NDVH sub-terreine word aangedui as wesenlik eenvoudiger as dié in natuurlike areas asook verskillend in samestelling. Die herstel van bestuiwing as 'n hoeksteen in na denneboom-aangeplante gebiede staar 'n aansienlike uitdaging in die gesig, gegee die versteuring van grond wat dekades van denneboom aanplanting gevolg deur denneboom bos brand kan veroorsaak. Sommige voorstelle is gemaak vir die herstel van fynbos gebiede wat herstel van denneboom aanplanting, insluitend 'n bespreking van die aanvulling van plantegroei hervestiging maatreëls (fynbos saadverspreiding en saailinge plant) met die metodes van die herstel van 'n gesonde pedogenese, bogrondse geleedpotige gemeenskappe, en fynbos saadbanke.

**Acknowledgements**

This project was funded by the CIB DST-NRF Centre of Excellence for Invasion Biology and Stellenbosch University.

That said, there are so many individuals who have sacrificed so much to help me with this project and "Whoever is not grateful to people, has not shown gratitude to God." - Prophet Muhammed (p. b. u. h.)

I could never, *ever*, have accomplished this task without the unfailing and kind support of Pr. Michael Samways. Dr. Corey Bazelet has also been extremely patient with me in all of the ups and downs of my research and has been of absolutely crucial help in completing this thesis with considerate advice and a sympathetic ear. Dr. Morne Lamont was extremely helpful with statistical advice and coaching.

It goes without saying, that the patience of my friend and companion, Atiyya, has been likewise a source of support and guidance as well in this project. My children became "research orphans" in the process of this work and I will feel guilty about the neglect of their primary right to a present father.

Without a shadow of doubt, my Mom and Dad were always present giving encouragement, moral support, and financial assistance during this arduous process. Both my brothers were always there as well to encourage. I thank them all them from the bottom of my heart.

All the support staff, Colleen, Marlene, Adam, Monean, and Fundiswa were of wonderful assistance. Dr. Connal Eardley of the Plant Protection Research Institute in Pretoria was most helpful and generous in his identifications of bees and in his advice on my bee specimens and species. Kind thanks are due to him for his dedicated work. Prs. Henk Geertsema and Pia Addison provided foundational advice and training in bee identification and are thanked kindly. Luther Van der Mescht is thanked for translating the general summary into Aafrikaans. Jannie Groenewald offered outstanding field assistance and excellent plant identifications and I thank him profusely. Pr. E. G. H. Oliver generously identified the *Erica* species. Dr. Dorrat-Haaksma, Dr. Manning, and the staff at Compton Herbarium are all kindly thanked for being patient in trying to identify my "eco-scraps" to the best of their expertise. I was lucky to bump into Dr. Tony Rebelo, in the hall who stopped his busy schedule to step into my cubicle and have a look at my Proteaceae "eco-scraps" to identify them. My heartful appreciation goes to Sthembiso Abdullah Gumede who was of crucial help in organizing specimens and collating data during many hours of mind-numbing lab work. Colin Tucker and Mr. Granger are also thanked for their field assistance. Martina Treurnicht provided beneficial initial vegetation ecology advice (I only realized much later how valuable it was). My highschool fieldworkers need to be mentioned as well: Dullan (no. 1), Dullen (no. 2), Curtwin, Keanan, Edwin, and Enrico came through for that vital late December 2010 field work that forms the crux of the data here.

In the final stages of my work two reviewers (who wish to remain anonymous) were kind enough to read over the manuscript and offer valuable input are also owed their due acknowledgements.

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# Chapter 1 – Main Introduction

*Present-day environmental crisis*

The greatest threat to biodiversity today is the loss of suitable habitat for organisms (Primack 2010, Hunter & Gibbs 2007) followed by the threat of invasive species (Cao *et al.* 2014, Cheng *et al.* 2009, Picker & Griffiths 2011, Sujay *et al.* 2010, Vitousek *et al.* 1997). The International Union for the Conservation of Nature (IUCN) Red Data List categorizes 21,286 species of 71,576 assessed species (30%) as being under threat from extinction (IUCN Red Data List 2014). In such an anthropogenic environmental crisis there is much pressure for solutions and proposals to be deal with each of the many threats. The route the conservationist takes varies much – depending on the scientific (Cassey *et al*. 2005), vocational (Maddock & Samways 2000), linguistic (Lowry *et al.* 2012, Rejmánek *et al.* 2002), philosophical (Rolston 1975, Woods & Moriarty 2001), historical (Maddock & Samways 2000), geographical (Lowry *et al.* 2012), psychological (Samways 2005b) and ethical (Brown & Sax 2005, Cassey *et al.* 2005, Samways 2005b) outlook.

Biodiversity is important, as our sources of food, fibre, and fuel are all biologically derived. Human-bred monoculture sources of these necessities are, however, susceptible to harmful biotic and abiotic pressures occasioned by global warming, invasive organisms etc. Naturally biodiverse areas provide a gene-pool resource for plant breeding to incorporate resistance to such stressors as well as a source of organisms for biocontrol (Hunter & Gibbs 2007, Primack 2010). Such human-undisturbed natural areas provide the full, bewildering breadth of ecosystem processes, dependencies, and interactions that have acted uninterrupted for all biological history with the exception of a few natural global mass extinction events characteristic of the present Anthropocene (Crutzen & Stoermer 2000) mass extinction event. Because species are honed often over millennia to produce those that endure the competition of survival of the fittest (Darwin 1903), conserving biodiversity for its direct utilitarian value therefore means conserving evolutionary processes as well (Hunter & Gibbs 2007, Richardson *et al.* 2001).

Biodiversity is also of utilitarian value because of the economic value of ecosystem services provided by natural areas to humans. Despite serious differences of opinion as regards to how to define such ecosystem services (Fisher *et al.* 2009), such benefits of biodiversity have been estimated at US$ 16-54 trillion ($10^{12}$) per year, with the average of various estimates coming to US$ 33 trillion per year (Costanza *et al.* 1997). However, such figures themselves are controversial because biodiversity value transcends the utilitarian to the aesthetic, cultural, and spiritual, and beyond even this to the intrinsic (Samways 2005a). Because of this, biodiversity monetization has its opponents (Pearce & Seccombe-Hett 2000) and its proponents (Odling-Smee 2005), both of whom ironically argue their case for the same stated reason: to save biodiversity.

*Restoration ecology*

A simplified sequence of conservation priorities is firstly to preserve and protect natural areas, secondly to implement ecologically conscious management systems in human interventions, and finally to restore transformed and damaged ecosystems (Hobbs & Harris 2001, Samways 2005a). However, for the half of humanity who live in highly populated and completely transformed urban and city areas (World Health Organization (WHO) 2009), restoration improves biodiversity interaction by reintroducing biota that are rich in comparison to those of non-enriched urban areas (Dobson *et al*. 1997). This revival of wildlife experience plays an important part in educating the public (Miller 2005, Samways 2007, Stokes 2006) as long as such restoration effort is contextualized within a balance between the actual needs of biodiversity and those of people (Elliot 1997, Heller & Zavaleta 2009, Holmes *et al.* 2012, Kull *et al.* 2011, Moll 2011, Pakenham 2007, Pfadenhauer 2001, Swart *et al.* 2001, van Wilgen 2012, van Marwijk *et al.* 2012). One such attempt at finding the balance utilizes Social-Ecological Systems (SES) analysis (Bettencourt & Kaur 2011, Carpenter *et al.* 2005, Ericksen 2008, Liu *et al.* 2013, Pollard *et al.* 2014).

Ecological restoration is perhaps best defined initially in a strict sense as an attempt to return a system to some historical state (Falk *et al.* 2006) – something which is generally recognized as difficult or impossible to achieve (Falk *et al.* 2006), and hence its placement as the last among the hierarchy of tools for conservationists as mentioned above. Restoration can be defined as the active efforts performed by restoration ecologists to effectuate an (hopefully well-defined) ecological goal and in this sense "recovery" can be differentiated from this as the actual response of ecosystems to such efforts. In this sense, "Restoration" is differentiated from "Restoration Ecology" which is the science of developing theory to guide such restoration and the science that uses such restoration to develop ecological knowledge (Falk *et al.* 2006). In the context of the research area under study in this thesis no active restoration effort was performed (not infrequently an actual intended ecological restoration strategy) other than systematic clearing of the area from re-growth of invasive tree species. For this reason I use the term "recovery" in this thesis as it more effectively implies the passive nature of maintenance in the area studied. The term "restoration" will be contextualized as it is used to avoid confusion.

*Restoration ecology in the context of invasive species*

While alien organisms are among the most immediate threats to biodiversity today (Mack *et al*. 2000, Picker & Griffiths 2011, Pimentel *et al.* 2000, Vitousek *et al.* 1996, 1997), they are also of importance to many humans economically (Devendra 1981, Maredia & Eicher 1995, Pimentel *et al.* 2000, 2005, South African Forestry Company Limited (SAFCOL) 2013). As well, few extinctions have been rigorously proven to be caused solely by invasive organisms (e.g. extinction of vertebrates in Guam due to introduction of the brown tree snake *Boiga irregularis*) (Gurevitch & Padilla 2004), and much debate continues about the attitude people should have to non-indigenous organisms (Brown & Sax 2005, Cassey *et al.* 2005, Davis *et*

*al.* 2011, Kull *et al.* 2011, Simberloff *et al.* 2011). Nevertheless, there is no doubt that lower-level biodiversity threats interact synergistically to create large threats and challenges to conservation (Brodie *et al.* 2012, Underwood *et al.* 2009) and it is generally accepted that these synergistic processes make invasive species the second biggest biodiversity threat after habitat destruction (Breithaupt 2003, Riley 2009, Sujay *et al.* 2010, Vitousek *et al.* 1996, 1997) (arguably the third after climate change).

Can ecological restoration address the threats of invasive species? In the context of South Africa this question has particular importance as the conservation efforts here face invasive species as one of the most important arenas in the maintenance of its valuable biodiversity.

*Conservation focus: the Cape Floristic Region as a South African biodiversity Hotspot*

The Western Cape Province of South Africa has within it one of the world's six floristic kingdoms, the Cape Floristic Region (CFR), mostly of fynbos, characterized by evergreen sclerophyllous heath and shrubs dominated by Restionaceae, *Erica*-like plants (ericoid plants), with the most common larger plants being Proteaceae, but with few indigenous trees (Allsopp *et al*. 2014). Within the fynbos, several regions have been declared by the United Nations Environmental, Scientific and Cultural Organization (UNESCO) Man and the Biosphere (MAB) Programme as World Heritage site Biosphere Reserves (BRs) for biodiversity. These BRs include the Kogelberg BR (1998), the Cape West Coast BR (2000), and the Cape Winelands BR (2007) (Pool-Stanvliet 2013). It is one of only five regions in the world with a Mediterranean-type climate, having cool, wet winters and hot, dry summers. The CFR has some 9030+ species of vascular plants all within an area of 90 000 $km^2$ of which some 68.7% are endemic (Goldblatt & Manning 2002). This is similar to the plant diversity in the wet tropics (Goldblatt & Manning 2002). The plant diversity of the CFR represents some 20% of plant species in Africa while only occupying about 0.5% of the African continent (United Nations Educational, Scientific, and Cultural Organization (UNESCO) 2014).

Of these 9030+ species, some 1805 species of plants listed in the IUCN Red List as Threatened Taxa (South African National Biodiversity Institute (SANBI) 2014) – the highest known concentration of rare plants in the world (Allsopp *et al.* 2014, Cowling & Hilton-Taylor 1994). The Western Cape is also globally distinguished by having extremely high β-diversity (Goldblatt & Manning 2002).

The Cape ecology is characterized by having a very high natural fire frequency (Allsopp *et al.* 2004, Cowling 1992), with fires typically occurring approximately every 15 to 20 years but with some significant variation from this depending on the specific fynbos area (Southey 2009). Increasing incidents of anthropogenic fires (via neglect, accident, or otherwise), a result of increasing populations, has significantly increased fire frequency above the natural fire frequency. This poses other conservation problems such as threats to certain species of plants not able to reach viable seed-producing stages in inter-fire intervals

(Syphard *et al.* 2009, 2007, van Wilgen *et al.* 2010). However, this increased fire frequency threat actually only aggravates the by far largest conservation threats to the Cape area which come in the form of habitat transformation due to invasive species, agriculture, and urban development (Rouget *et al.* 2003).

This combination of high endemicity under threat is what originally motivated Myers (1990) and Myers *et al.* (2000) to classify the CFR (and other areas in the world) as a "hotspot" of biodiversity, though concerns have been raised about this classification being used as an absolute for conservation prioritization efforts (Kareiva & Marvier 2003, Possingham & Wilson 2005).

*Invasive organisms in the Western Cape*

Within the threat of invasive organisms, invasive plants are the greatest threat to CFR biodiversity (Gaertner *et al.* 2009), with invasive trees and shrubs being the most significant in transforming and disturbing natural landscapes (Rouget *et al.* 2003). Of these, pine trees are particularly important as they are among the most (if not *the* most) invasive of the invasive trees (Richardson 2006), while also being the most widely cultivated forestry trees in South Africa as well as elsewhere (Richardson 1998). Pine trees have been grown where they are not indigenous for commercial purposes (pulp, lumber) in South Africa, New Zealand, Australia, Chile, Brazil, Argentina, and other tropical and subtropical regions (Le Maitre 1998, Richardson 1998).

In areas where they are managed in the Western Cape, pine stands shade out virtually all other vegetation, leading to a localized extinction event for the large majority of plants on the forest floor (Figure 1.1). Fynbos, the dominant natural vegetation of the CFR, has few indigenous trees, meaning that wherever pine trees are grown in managed stands, there is little fynbos left. As a consequence, most of the normal fynbos ecosystem also becomes functionally extinct in managed pine stands, and this includes animal-mediated fynbos pollination, animal-mediated fynbos seed dispersal (with the exception of dispersal by Cape Baboons, *Papio ursinus ursinus*), and most animal-fynbos plant interactions.

(Figure 1.1 here)

Although conservation effort in the Western Cape is maintained in larger national, provincial, and municipal nature reserves, conservation of insect-plant interactions and most importantly, insect-mediated pollination, transcends the "large reserve" focus of conservation. This is because pollination and pollinator conservation brings conservation into agro-ecological systems.

Pines do not need insect pollinators because they are wind-pollinated (anemophilous). Fynbos, by contrast, has a majority of species reliant to some extent on faunal mediated pollen vectors (Allsopp *et al.* 2014). Recent anthropogenically increased fire regimes have caused huge losses to the pine forestry industry. Such economic loss has forced forestry stakeholder interest to reconsider the wisdom of converting

areas of fynbos to pine stands for economic gain. A huge fire in 1999 in what was then the Haweqas Provincial Nature Reserve destroyed pine forest and surrounding fynbos. Following this fire an agreement was ratified to turn the Zachariashoek plantation into a fynbos restoration area. These events turned this area into a perfect research opportunity to compare how vegetation and pollinator communities (focusing on bees) in the same post-fire successional stage, recover in previously pine-afforested areas (hereafter referred to as post-pine recovery areas – PPR areas for short) vs. those which always contained natural vegetation. These latter areas are referred as Natural though it is understood that there were edge effects in this area to due pine trees and that these may continue even after the pines have been extirpated (this will be explained further in Chapter 2).

*Objectives of this study*

(1) To gain an understanding of how common fynbos plants in the same post fire-succesional stage compare between Natural areas and Post-Pine Recovery (PPR) areas (Chapter 2);
(2) To develop appropriate methodologies for sampling bee assemblages in the fynbos (Chapters 3 and 5);
(3) To compare bee diversity, abundance, and composition between Natural and PPR areas of the reserve (Chapter 4); and
(4) To make general inferences on bee pollination web comparison between Natural and PPR areas based on (1) and (2) above, and to make management recommendations for recovery of disturbed fynbos sites (Chapter 6).

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Figure 1. 1 Two different ecological contexts can give two diametrically opposite conservation evaluations. In its own biological origin (left – photo kind permission of Dida Kutz), *Pinus radiata* D. Don, is classified as Endangered (EN) by the IUCN in an old growth forest from Point Lobos State Natural Reserve, SW California. In the South African context the same species ironically becomes ferociously invasive but is nevertheless silvicultured there for timber (right – a cultivated *P. radiata* forest from Wemmershoek, Western Cape). Notice the presence of understory vegetation (and associated understory ecosystem) adapted to survival under pine canopies and the complete absence of the same from the cultivated forest – an effective local fynbos extinction event (though there is surviving fynbos seed under layers of comparatively heavy *Pinus* litterfall (Cilliers *et al.* 2004)).

# Chapter 2 – Recovery of vegetation after removal of pine forests by fire in the Limietberg area of the Cape Floristic Region biodiversity hotspot


## Abstract

The Cape Floristic Region (CFR) in the Western Cape (WC) Province of South Africa has the highest biodiversity of all Mediterranean-type climate regions globally. Invasive plant species are a major threat to biodiversity in the region, while afforestation with alien pine trees (*Pinus* spp.) is a further threat. In 1999, an accidental wildfire in the CFR burned much of the Limietberg Nature Reserve, along with an adjacent pine tree plantation which was then left to recover to the native fynbos vegetation. I compared species abundance, species richness and species composition in areas which had been pristine fynbos prior to the fire and areas which had been afforested with pines prior to the fire using a paired experimental design. Twenty quadrats were placed along eight 40 m transects, each of which was placed parallel to the natural vegetation ('Natural')/Post-pine recovery (PPR) boundary in the Natural and the PPR sub-sites. Transects were positioned at eight distances from the boundary such that five transects were within the 50 m ecotone region and three transects were deep (60 to 100 m) within the Natural and PPR sub-sites, respectively. Natural sub-sites consistently had higher total species abundance and species richness than PPR sub-sites (Natural: 126 species; PPR: 88 species; excluding 27 geophyte-like and other unidentified species). No significant correlation in species richness or abundance with distance from the Natural/PPR boundary was present for either PPR or Natural sub-sites. Half of the plant species abundance observed in the reserve differed significantly between Natural and PPR sub-sites. Of these, about two-thirds were more abundant in Natural sub-sites and one third was more abundant in PPR sub-sites. *Erica* spp., *Restio* spp., and *Hypodiscus* spp. were more abundant in Natural sub-sites, while *Helichrysum* spp. were more abundant in PPR sub-sites. These results show that pine afforestation of fynbos continues to impact vegetation assemblages even 11 years after removal by a fire event. Although both Natural and PPR sub-sites were left to recover, their successional trajectories differed. I hypothesize that this is a result of disturbance to soil structure and chemistry caused by a lengthy history of pine forest shade and litterfall followed by a subsequent accidental pine forest fire during the hot/dry season.


## Introduction

The present environmental crisis, in which some 30% of assessed species are categorized as under threat of extinction (IUCN 2014), requires major focus on threats to plants, because plant life forms the foundation of ecosystem trophic levels, being the primary autotrophs upon which all life depends. Furthermore, conserving plant biodiversity means conserving plant species in their role in ecosystem processes, and since all ecosystem processes are evolutionary, this means conserving evolutionary processes as well (Hunter & Gibbs 2007, Richardson *et al.* 2001, Primack 2010).

This crisis is important to alleviate not only for biodiversity's aesthetic, cultural, and spiritual value (Samways 2005) but also for economic reasons as areas that are home to such diversity perform valuable ecosystem services which has been assessed in one study at US$ 16 to 54 trillion ($10^{12}$) (Costanza *et al.* 1998).

The Western Cape Province of South Africa has within it the fynbos heath and shrublands and is one of the world's six floristic kingdoms, the Cape Floristic Region (CFR). The CFR contains 9030+ species of vascular plants, of which 68.7% are endemic to the CFR (Goldblatt & Manning 2002). This represents 20% of plant species in Africa, while only occupying about 0.5% of the African continent (United Nations Educational, Scientific, and Cultural Organization (UNESCO) 2014).

The CFR has the highest concentration of threatened plants in the world with 1805 species of plants listed in the IUCN Red List as Threatened Taxa (South African National Biodiversity Institute (SANBI) 2014). The combination of high endemicity in the CFR along with major threats to it from habitat transformation of various kinds (Rouget *et al.* 2003) motivated Myers (1990) and Myers *et al.* (2000) to classify the CFR as one of the global "hotspots" of biodiversity.

Of the threats listed by Rouget *et al.* (2003) to the fynbos, invasive species are the second most serious after direct habitat transformation from agriculture and other human development. Among the threatening invasive organisms, invasive plants are the greatest threat to CFR biodiversity, with invasive trees and shrubs transforming and disturbing the natural landscapes (Gaertner *et al.* 2009, Rouget *et al.* 2003). Of these, pine trees (*Pinus* spp.) stand out as they are among the most invasive of the invasive trees (Richardson 2006) as well as being the most widely cultivated forestry trees in South Africa and elsewhere (Le Maitre 1998, Richardson 1998).

In areas where pine trees are grown in the CFR they shade out virtually all other vegetation, leading to a localized extinction of many plants on the forest floor (Fig. 1.1). Fynbos, the most widespread natural vegetation of the Western Cape (Allsopp *et al*. 2014), has few indigenous trees so wherever pine trees are grown in managed stands, little fynbos remains. Consequently, most of the natural fynbos ecosystem also becomes functionally locally extinct in managed pine stands, and this includes animal-mediated fynbos pollination, arthropod seed dispersal (though there is some dispersal by Cape Baboons, *Papio ursinus ursinus*, and possibly some other larger mammals), and most animal-fynbos plant interactions.

Naturally occurring fire is integral to fynbos ecology, with an estimated burn frequency of 15 to 20 years with possibly some variation from this depending on the actual fynbos area (Southey 2009). A number of fynbos plants have traits such as seeds stimulated to germination by smoke *per se,* pyriscient (and pyrohydriscient) serotiny, post-fire resprouting, etc. that give them advantages for survival in areas with such a comparatively high burn frequency (Brown 1993, Keeley *et al.* 2011, Montenegro *et al.* 2004, van Wilgen *et al.* 1992). Unfortunately, even such traits may not be sufficient to protect certain species under a burn

frequency that is now anthropogenically even higher than the already high natural fire frequency (Syphard *et al.* 2009, 2007, van Wilgen *et al.* 2010).

Fire in pine forests in the CFR tend to burn much hotter than natural fynbos (Brooks *et al.* 2004). Soils that are subjected to extremely hot fires lose some capacity to absorb water, which leads to erosion in post afforestation areas (DeBano 2000, Scott & van Wyk 1990, 1992).

Removal of pine tree stands eliminates a significant invasive species propagule source, and this is the first step taken in restoring pine afforested areas in the CFR to fynbos. The question then arises, how much biodiversity returns in areas afforested by pines and subsequently restored to fynbos? What is the conservation value of species recovering in restoration areas? Basic information on the recovery of fynbos from pine tree afforestation is important to make informed management decisions regarding pine tree stands, to design policies for mitigation of negative effects of pine trees, and for designing informed plans for fynbos restoration after pine tree removal.

In this chapter, I ask whether species richness, abundance and composition of herbs and bushes between two areas burned in the same fire event, one which was natural fynbos prior to the fire and the other which was afforested with pine, converge following fire. I then draw conclusions regarding the effects of extensive pine forest shading and pine tree litterfall followed by a probably hotter-than-usual pine forest fire, on recovering fynbos vegetation.

## Methods

### *Study area*

This study was conducted at Zachariashoek, Haweqas Nature Reserve (part of the larger Limietberg Nature Reserve), Western Cape, South Africa, (33.8291 S, 19.0518 E) (Fig. 2.1). The area has a rugged terrain with steep slopes and deep valleys and is an important river catchment area for the Wemmershoek River, all within the Cape Winelands Biosphere Reserve (CWBR). Until 1999, the valley and inner mountain slopes of Zachariashoek were afforested with Monterey pine trees (*Pinus radiata* D. Don) under the management of a private forestry company, Mountains to Oceans Forestry (MTO) (Pty) Ltd. The area of pine afforestation had been under management for pine forests (for maximum lumber and pulp yields) for 40 years. The surrounding area was a Nature Reserve with a natural fynbos ecosystem under management by CapeNature, the Western Cape provincial conservation body, which removed unmanaged pine tree growth resulting from escaped propagules ('opslag pine') encroaching outside of the demarcated forestry area. In 1999, a major accidental summer fire swept through the entire valley, burning down the pine forest and the surrounding reserve fynbos matrix. This fire was hotter than fynbos fires in the CFR because invaded areas typically have higher fuel loads, which results in hotter fires (Brooks *et al.* 2004). Additionally, some

circumstantial evidence strongly indicated a very hot fire – even hotter than pine forest fires deliberately lit in the winter (see discussion below). After the fire, the area was not replanted with pines and a management contract was concluded with CapeNature to allow Zachariashoek to undergo seral succession into a fynbos reserve area.

This burn event enabled a direct comparison of post-fire vegetation between two areas with different biotic histories that were both in the *same* post-fire successional stage. One area was a post pine recovery (referred to here as 'PPR') area and the other area had contained natural fynbos vegetation prior to the fire and is hereafter referred to as 'Natural'. Having the same post-fire successional stage (restored, natural or otherwise) is important for comparison of vegetation in different fynbos areas as the composition of fynbos vegetation is strongly affected by the time elapsed since the last burn (Capitanio & Carcaillet 2008, Cowling 1992, Kruger & de Bigalke 1984, Southey 2009).

*Study sites*

The study area encompassed five replicate sites, each consisting of adjacent, paired Natural fynbos and PPR sub-sites, in the Zachariashoek valley in the southwestern corner of the Limietberg Nature Reserve (Fig 2.1). All sites were in the Haweqas Sandstone Fynbos biome (FFs 10) (Mucina & Rutherford 2006). Each Natural sub-site was paired with an adjacent PPR sub-site, and the transition between the two is referred to as the Natural/PPR boundary (Fig. 2.2; for photos of research sites see Appendix 4). The sites were chosen in a way that both PPR and Natural sub-sites had the same elevation, topography, and aspect. Grid references, elevation, aspect, and slope details of the sites are given in Table 2.1.

(Figure 2.1)

(Table 2.1)

*Vegetation sampling*

Sampling was between mid-March and mid-May 2011, and was done using transects. Each transect was performed using a 20 m rope with tags tied every 2 m. Either end of the rope was attached to stout vegetation. A plastic ring with a diameter of 20 cm was used as a quadrat to sample vegetation every 2 m along the transect rope. The ring was positioned with the 2 m tag centred in its middle and a stout wooden pole was then positioned at its edge at an angle perpendicular to the slope of the immediate vicinity. The pole was held firmly in position and all vegetation was viewed from above the ring. With the pole held firmly, the ring was gradually moved down to the ground using the pole as a guide (Fig. 2.2). Plants either touching or within the volume of the "virtual cylinder" delimited by the ring were recorded as present for that quadrat. Plants were identified where possible to the species level in the field or sampled and given

morphospecies names to distinguish them. To avoid pooling separate species together, any minor variations in plant structures were noted with new morphospecies names and observations being kept as consistently as possible according to such classifications. Small quadrats are problematic for sampling large plant species (e.g. trees and large bushes) (Stohlgren 2007) so plants were also classified into one of three groups according to their size for verification of sampling sufficiency with Species Accumulation Curves (SACs): species that were never locally taller than 40 cm ('size A'), species that were between 40- 140 cm ('size B'), and those that were locally observed to grow taller than 140 cm ('size C').

(Figure 2.2)

*P. radiata* trees commonly propagate seed up to a distance of 100 m from the pine forest edge (Richardson *et al.* 1994) and edge effects can also, theoretically, reach up to 100 m (Harris 1988, Risser 1995, Young & Mitchell 1994). However, such was not to be expected in the case of Zachariashoek, as the area around the pine forests had been managed and cleared regularly of wild-growth pine by the local conservation authority, CapeNature. This is further corroborated by other research that shows pine trees to have edge effects that drop off greatly after 32 m (Pryke & Samways 2012). To be conservative, I made Ecotone zone 40 m wide, to account for pine dispersal behaviour. I refer to transects farther than 60 m from the boundary as Deep zones. From a simple visual inspection the comparative topography between Natural and PPR sub-sites was noticeably different going farther than 100 m into either Natural or PPR sub-sites for all five sites (except one).

Eight transects were placed parallel to the Natural/PPR boundary in each of the Natural and PPR sub-sites. Excluding Site 3, the average slope was about 37° (see Table 2.1) which was a significant obstacle in collecting vegetation data on foot that was further impeded by numerous large boulders, 3 to 15 m cliffs, and often very dense, coarse, and thorny vegetation. For this reason each transect was established as two parallel 20 m transects separated by two paces (1.6 m), referred to as '20 m transect pairs', to make vegetation data sampling possible at these sites under the time constraints of the study. This process was adopted at Site 3 for procedural uniformity even though terrain at Site 3 was significantly less steep and rugged.

These 20 m transect pairs are hereafter referred to as '40 m transects' to avoid confusion with the statistical pairing of transects between Natural and PPR sub-sites for statistical comparison. Each 20 m "half" from these 40 m transects had 10 evenly spaced sample points along it totalling 20 sampling points per 40 m transect. Five 40 m transects were positioned within 50 m of the Natural/PPR boundary to measure edge effects (Ecotone samples) – at 3, 10, 20, 30, and 40 m from the boundary, respectively. Three 40 m transects were positioned between 50 m and 100 m from the boundary (Deep samples) – at 60, 80, and 100 m from the boundary (Fig. 2.2). No comparative analysis was possible after 100 m due to the sharp differences in landscape, such as precipices of 20 m + and proximity to riverine areas.

From eight 40 m transects there were 160 sample points in total for each Natural and PPR sub-site. Each site thus had 320 sample points for a total of 1 600 sample points (see Fig. 2.2 for photographs and a schematic diagram) for all 5 sites. The total sampling area of these 1 600 analysis "points" covers 1 600 × $\pi$ × radius$^2$ which equates to 50.27 m$^2$ in total, 10.05 m$^2$ per site, or 5.03 m$^2$ for each sub-site.

It is a common protocol for sampling of herbs and small bushes together to use a square 1 m by 1 m or larger quadrat and to only count as present plant species that enter the soil within the quadrat (Stohlgren 2007). Small quadrat size and transect methodology are known to be effective for studies of plants common to the study area (Stohlgren 2007) and this is sufficient for a study of the scope of this one. Such small quadrats do, however, seriously underestimate species accumulation curve extrapolations for numbers of species for assessment of taxonomic sampling "completeness" (Stohlgren 2007, Barnett & Stohlgren 2003), being only sufficient for estimates of locally common species (Stohlgren 2007). This is particularly so for patchy vegetation types (Stohlgren 2007), something for which fynbos is notorious (K. J. Esler, personal communication), and so here I opted for more, smaller quadrats appropriate for the fynbos where understory plants were also included.

Trees and large bushes (size group C) could not be included in analyses in the same way as their smaller counterparts because of the inherent bias in the sampling methodology. Therefore, these data were kept separate throughout the main analyses.

*Plant identification and categorization*

Plant specimens were collected for each recorded species and morphospecies and, where possible, were identified in the field. Remaining specimens were given field names and later identified by experts. In the case of plants which were inconsistently distinguished in the field, data were pooled (Table 2.2). Also, as some plants did not have distinguishing features such as flowers (as in the case of some geophytes), they could not be identified to the species level. In total, 27 plants could not be identified to family level, and they are listed in Appendix 2 as Morphospecies 1, Morphospecies 2 … etc.

(Table 2.2)

The fynbos classifications/definitions are not universally agreed upon (proteoid, ericoid etc.). To simplify classification, G (Graminoid - including Poaceae, Cyperaceae, Restionaceae) was used as the main classification group, and thereafter everything was grouped according to leaf size and shape. Plants with small (never longer than 25 mm) and thin (width never more than about a third of the length) leaves were grouped as E (Ericoid). Longer and/or wider leaved plants were grouped as F (Flat leaved). The few geophytes that were blooming were separately grouped as Bu (Bulbs). All other plants that had leaves that

did not fit into these groups were pooled as O (Other). I finally classified vegetation according to family and IUCN status (IUCN Red List 2014) for subsequent analyses according to these categories.

*Other observations*

General observations ("first glance" observations) on disturbances such as erosion and larger plant species appearing to be dominant, conspicuously absent, or obviously more lushly or vibrantly growing were made and recorded (Table 2.3).

(Table 2.3)

*Statistical analysis*

Species accumulation curves (SACs) were generated using EstimateS Version 8.2.0 (Colwell 1997) to assess sampling sufficiency. After removing species with three or less occurrences, STATISTICA 11 (StatSoft, Inc. 2012) was used to compare differences in species richness and abundance between PPR and Natural fynbos sub-sites across all distances from the boundary using paired t-tests, one-way and two-way ANOVAs, and non-parametric equivalents. Wilcoxon's matched pair t-tests were used when subsets of data violated the assumptions of parametric statistics (i.e. were not normally distributed). Eight separate Wilcoxon's paired t-tests were used to compare species richness at each of the eight distances for each pair of sub-sites (Natural or PPR) at each of the five sites for trees and large bushes that had sufficient non-zero data pairs (6 or more). The smaller plant size groups (A and B) were also analysed to see how such richness statistics compared with Wilcoxon paired t-test statistics of abundance data. Finally, I performed linear regression analysis to evaluate changes in vegetation patterns with distance from the boundary.

If any genera had three or more species, data were also pooled for these and a Wilcoxon's paired t-test was performed on these pooled data sets to estimate differences in plant abundance between Natural vs. PPR for each genus.

All locally rare species for which there were three or less occurrences were pooled and examined together. A Wilcoxon's paired t-test was used to see if there were any differences between the PPR and Natural sub-sites for the rare species as a group.

## Results

*Raw biodiversity*

A total of 175 different plant morphospecies were collected. Of these, 27 morphospecies could not be identified even to family level. Of the species collected, 87 species were listed on the IUCN Red List as LC (Least Concern), two as VU (Vulnerable), and one as NT (Near Threatened).

There were 71 shared plant species between Natural and PPR sub-sites. Additionally, 55 species were exclusive to the Natural sub-sites and 17 species were exclusive to the PPR sub-sites (Fig. 2.3). Notably, 16 of the 17 species that were present solely at the PPR sub-sites were present in the deep region of the PPR sub-sites. In comparison, of the 55 species exclusive to the Natural sub-sites, 40 were in the Natural deep areas.

(Figure 2.3)

*Sampling Sufficiency*

Species accumulation curves were closest to asymptote for plant sizes A and for plant sizes A and B together (Fig. 2.4). I conclude that sampling effort was sufficient for size groups A and B as curves for these sizes showed clear non-linearity. However, when all plant sizes were included, the asymptotic nature of curve was less pronounced indicating inadequate sampling. This was expected as sampling method was deemed inappropriate for trees.

(Figure 2.4)

*Plant Diversity in Natural vs. PPR Sub-sites*

When all groups, geophytes, species complexes, and morphospecies which could not be identified to family level were removed, size group A species abundance was significantly higher in the Natural sub-sites than in the PPR sub-sites ($t = 2.69$, $P = 0.011$, Figure 2.5 i and Figure 2.5 ii). Species abundance in deep areas (>50 m from the boundary) of Natural sub-sites were compared with the deep areas of PPR sub-sites and here no significant difference was found for size A plants ($t = 1.78$, $P = 0.097$), although combining both plant size A and B showed higher abundance in Natural sub-site deep zones (>50 m) ($t = 2.81$, $P = 0.014$) (Figure 2.5 iii). When all groups, geophytes, species complexes, and morphospecies which could not be identified to family level were included, size group A plants were significantly more abundant in the Natural sub-site ecotone zone (<50 m from boundary) than in the PPR sub-site ecotone zone ($t = 3.74$, $P = 0.001$). However, removing the problematic plant groups indicated no such difference between the ecotones of the Natural and PPR sub-sites ($t = 1.99$, $P = 0.058$). Size group A and B plants together were significantly more abundant in Natural sub-site ecotone zones (<50 m) than in PPR sub-site ecotone zones ($t = 4.89$, $P < 0.001$)

(Figure 2.5 iv). There were no significant differences between deep vs. ecotone in the PPR sub-sites (paired $t = 0.65$, $P = 0.55$) or deep vs. ecotone in the Natural sub-sites (paired $t = 0.52$, $P = 0.63$) for the combined size group A and B.

(Figure 2.5)

There was significantly higher species richness of size group A plants in Natural sub-sites than in PPR sub-sites ($t = 2.48$, $P = 0.018$). However, there was no significant difference in species richness of size group A plants between Natural deep zones and PPR deep zones ($t = 1.70$, $P = 0.11$), or between Natural and PPR ecotones ($t = 1.90$, $P = 0.069$).

Species richness of size group A and B plants together was significantly higher in Natural sub-sites than in PPR sub-sites overall ($t = 4.88$, $P < 0.001$), as well as for Natural deep zones vs. PPR deep zones ($t = 3.64$, $P = 0.003$), and Natural ecotones vs. PPR ecotones ($t = 3.32$, $P = 0.0029$) (Figure 2.6 i, ii, and iii).

(Figure 2.6)

There was no significant interaction between site type (Natural vs. PPR) and distance class (two-way ANOVA $F = 0.010$, $P = 0.92$) (Fig. 2.7).

(Figure 2.7)

Linear regression showed no significant correlation between abundance or species richness and distance from the boundary (Fig. 2.8).

For individual species with six or more occurrences ($N = 50$) of non-zero data pairs, a Wilcoxon's matched pair t-test revealed significant differences between Natural and PPR abundances for 26 plant species/complexes. Of these, the largest group ($n = 10$) were from the Restionaceae, followed by the Proteaceae ($n = 6$) (Figure 2.9 i and ii), with the rest being an assemblage of bushes and herbaceous plants.

(Figure 2.8)

*Species Composition in Natural vs. PPR Sub-sites*

The 24 (±3) species for which there were significant differences, excluding grasses and sedges (Poaceae and Cyperaceae), is the median between the minimum possible number (21) of species and the maximum (27) due to the inclusion of two complexes, so involving some uncertainty. Restionaceae, Proteaceae, and the remaining group of various herbs and smaller shrubs are split evenly into species/complexes displaying significant differences and those that did not. Species displaying significant differences were mostly more abundant in the Natural sub-sites (Table 2.4).

(Figure 2.9)

(Table 2.4)

Species that were difficult to consistently differentiate in the field were pooled to form ten groups (Table 2.2). Of these ten, seven had sufficient non-zero data pairs to analyse with Wilcoxon's matched-pair t-tests and of these seven, two had t values indicating significantly higher abundance medians at the Natural sub-sites and one indicated a higher abundance median at the PPR sub-sites (Table 2.5 (a)).

(Table 2.5)

Data were pooled for genera which had three or more species and which were consistently distinguishable. Four of nine genera which could be analyzed (those with three or more species) were significantly more abundant in Natural than in PPR sub-sites, and only one was significantly more abundant in PPR sub-sites than in Natural sub-sites. Differences for the other four genera were not significant (Table 2.5 (b)).

Grasses and sedges comprised a total of 26 species. Family abundance of the two families Poaceae and Cyperaceae did not differ significantly in Natural and PPR sub-sites ($t = 223.5$, $P = 0.053$). The statistical tests on distribution of individual species and species richness comparisons in either family were not possible due to identification difficulties.

Species richness and abundance did not differ significantly between Natural and PPR sub-sites for Ericoid (E), Flat-leaved (F), Bulb/Geophyte – like (Bu), or Other (O) species groups, (data not shown). However, abundance of Graminoid (G) plants of sizes A and B in both ecotones and deep zones did differ significantly ($t = 1029$, $P = 0.0278$). This is attributed to the difference in Restionaceae between the sites, as grasses (Poaceae) and sedges (Cyperaceae) did not differ significantly.

Soil parameter measurements were not made. However, the soil was noticeably different in the two sub-sites. The soil in the PPR sub-sites was generally looser and than the Natural sub-sites, and showed evidence of erosion (Table 2.3). Visual observations showed that some plant species were noticeably more lush/vibrant in PPR sub-sites. Other larger plant species noticeably absent/present on either sub-site are also recorded in Table 2.3.

**Discussion**

Although Natural and PPR vegetation would appear similar with 71 species shared between them, this result is misleading, as comparison of abundances of those 71 species are frequently significantly disparate between the two sub-sites. Furthermore, Natural sub-sites had greater species richness than PPR

sub-sites. This was expected, since disturbed areas are often recolonized by indigenous but disturbance-adapted plants. Such plants can locally out-compete other native plant species decreasing such original native species' trajectories to the extent of even preventing them from recolonizing such disturbed areas without further active efforts to reintroduce/propagate native species (Reinecke *et al.* 2008). *Metalasia densa*, *Stoebe plumosa*, and *Stoebe cinerea* may qualify as such ruderal and locally invasive indigenous species due to their high density in colonizing PPR areas in Zachariashoek.

Different plant species can vary in their recovery time following disturbance owing to specific soil and micro-climate requirements, especially since soil often takes between 100 and 100 000 years to form (Targulian & Krasilnokov 2007). This is important in light of *Pinus radiata* causing disturbance to soil in at least three ways: (i) *P. radiata* litterfall is significantly higher than that of fynbos (Versfeld 1981, Richardson & van Wilgen 1986), (ii) *P. radiata* litterfall releases ethylene vapour inhibitive to fynbos species (Lill & McWha 1976), and (iii) pine-associated soils in Mediterranean-type climate areas have significantly lower rates of decomposition and depauperate microarthropod communities compared to natural soils (Springett 1976).

On the whole, this study agrees with the results of Richardson & van Wilgen (1986) who compared the effects of 35 years of afforestation on fynbos species and found a decrease in 58 % of the raw biodiversity from 298 spp. in 1945 to 126 spp in 1984. The pines in their study had been thinned by 30 to 50 % four times in the 35 yr period before the 1984 study, and had been pruned to 7 m once in that period as well. The *P. radiata* stand in my study had been actively managed for maximum lumber and pulp production which may or may not have been similar to this management strategy. Some speculations as to why there were differences (58 % drop in total number of species in post pine areas by Richardson & van Wilgen (1986) vs. 30 % in Zachariashoek) include: (a) differing vegetation sampling procedures, (b) differing post-fire successional stages, (c) differing ages of post pine recovery fynbos, and (d) there were some 27 plant morphospecies in my study and their spatial association is unexplored in my study due to various reasons (out of season sampling etc.).

Fynbos vegetation patterns are known to be sensitive to soil hydrology (Araya *et al.* 2011, Campbell 1986, Silvertown *et al.* 1999). Soil erosion was noticeably higher in PPR sub-sites (Table 2.3) and this is a well-known indicator of significantly compromised soil health (Acton & Gregorich 1995). Such differences are important in causing fynbos heterogeneity, and as a result, a species can be phenotypically different even over relatively short distances. Holmes *et al.* (2000) reported some differences in fynbos revegetation in the Noordhoek area for a Cape Peninsula Proteoid fynbos restoration project for different soil restoration regimes.

The question then arises, how well would the Natural and PPR sub-sites have been matched in the five sites of this study? Although within each of the five sites, PPR and Natural sub-sites were indicated as having the same vegetation type by Mucina & Rutherford (2006), no data on soil *micro*-differences between

sub-sites were available for this study. Soil hydrology between the PPR and the Natural sub-sites may have varied as equidistance of both sub-sites from streams (perennial, intermittent or otherwise) was not possible for all sites in this study. Nevertheless, the paired Natural and PPR sub-sites for this study were not in riparian areas and were *adjacent* with nearly identical slopes, elevations, and aspects for all five sites (Table 2.1). This, along with the fact that there are five replicate sites for comparison, should make the results of this study reliable if, on the whole, all other factors are assumed as equal.

A concerning finding was that, of the 17 species that were unique to the PPR sub-sites from this study's sampling protocol, 16 were present in the deep zones. This means that at 60 m or further into the PPR sub-sites, most of the common species are not the same common species as in the Natural sub-sites. This begs the question from where did they come? There are three, not mutually exclusive, possibilities here: (1) the seeds were present (buried under *P. radiata* originated soil) and managed to sprout and propagate after the fire; (2) the seeds were brought from afar (neither the PPR sub-sites or the Natural sub-sites) by the wind, birds, or even by baboons/large mammals; and/or (3) plants indicated as absent from the Natural sub-sites were actually there but undetected by my sampling protocol, and these unrecorded plants produced the seeds.

Fifty-five species were recorded only in the Natural sub-sites. Total absence or low level of occurrence of these species in the PPR sub-sites may be the result of competition from disturbance-adapted species, soil conditions being prohibitive for successful growth, or some combination of the two (see further discussion below).

Figures 2.5 and 2.6 show that, overall, PPR sub-sites were significantly disturbed in comparison with Natural sub-sites, with lower levels of both plant abundance and species richness. Half of Restionaceae species were sensitive to the differences between PPR and Natural sub-sites (this half being about evenly split – some preferring the Natural sub-sites and some preferring PPR sub-sites). Data for the other half of Restionaceae species showed no such sensitivity, with species showing no statistical preference for either sub-site. Half of the Proteaceae species were more abundant at the Natural sub-sites but there was no significant difference for the other half indicating that Proteaceae may or may not be sensitive to differences, but if there is sensitivity it is most likely to favour the Natural sub-site. Likewise, half of the assemblage of miscellaneous smaller bushes and herbaceous plants showed some sensitivity to Natural vs. PPR sub-site conditions. Of this half, about two thirds were more abundant at Natural sub-sites and one third in the PPR sub-sites.

The data do not reflect geophytes due to the timing of the plant study, but it seems highly likely that geophytes, for the same reasons mentioned above, might be more abundant or species rich in the Natural sub-sites. If all plant species, regardless of family or grouping, are pooled, the overall result is that about half of the species showed no sensitivity or preference to PPR or Natural sub-sites. From the other half, about two-thirds preferred the Natural sub-sites and one third preferred the PPR sub-sites (Table 2.4). This

overall result is similar to the overall result for the Proteaceae suggesting Proteaceae as an indicator family for fynbos overall plant recovery.

*Causes of soil disturbance*

All this begs the question what caused the soil to be so disturbed and why was there not a natural succession of plant species?

The answer to this is complex but it probably involves a combination of soil disturbance due to the synergy of three processes: (i) the epigaeic extinction of fynbos plant life in the shade of the pines and concomitant extinction, to some extent, of endogaeic plant life in the seedbank and plant root structures, (ii) the high annual litterfall of pine stands (with mature pine stands nearly doubling that of mature montane fynbos (Versfeld (1981), as reported by Richardson & van Wilgen (1986)) which could have buried fynbos topsoil (and seedbank) and prevented it from receiving typical stimuli for germination (this has been suggested to occur for fynbos seedbank under the litterfall of another invasive tree, *Acacia saligna* (Holmes & Cowling 1997)), and (iii) the probably hotter-than-usual nature of the fire in the pine forest area compared to the 'normal' fire of the fynbos matrix. The possible interaction of these three factors would require soil science, soil hydrology, and other specialist disciplines to resolve which of these factors or what proportions of them cause the disturbance.

One aspect that distinguishes the accidental 1999 Zachariashoek fire from other, deliberately lighted, pine forest fires (e.g. the burn standing fire treatment mentioned in Holmes *et al.* (2000)) is that the Zachariashoek fire was likely significantly hotter. This is because deliberately lighted fires invariably take place in the cooler and wetter winter months (for control reasons) during low wind conditions for various management purposes and are therefore less intense and severe than accidental hot and dry season fires. Although fynbos fire in the natural matrix around the forest was at the same time as the fire in the pine forest, the pine forest fire was hotter than these areas as fires in invaded areas typically burn at a higher temperature than non-invaded areas as a result of a higher fuel load (Brooks *et al.* 2004). Importantly, there is a (good) possibility that it was in fact much, much hotter in the area of the pine forest (or in parts of this area) than the usual type of fynbos fire in the fynbos matrix around the forest stand. I base this on anecdotal observations of pine stumps burned right down to the level of the ground with even the endogaeic root bulb of the tree being charcoal to 10 cm beneath the level of the ground at Site Five. Another piece of evidence is a melted gearbox of a vehicle that was trapped in the fire in the valley and is presently displayed as a memento on the Limietberg Reserve Manager's office wall – which strongly implies extreme heat as the melting point of iron is 1538ºC and the melting point of aluminium is 660ºC. Effects of such extremely hot fires include killing seedbank, plant bulbs etc. at a significantly deeper level than normal fynbos fires and possibly to disturb the soil to an extent greater than that engendered by the pine forest shading and litterfall alone. A further confounding factor is that the pine trees' high litterfall might have shielded the original

fynbos soil from heat if it did not burn completely in the fire as such unburned litterfall is an excellent thermal insulator (Neary *et al.* 2005). Such thermal protection has been suggested to occur for fynbos seedbank under the invasive tree, *Acacia saligna*'s litterfall (Holmes & Cowling 1997). Given that the fire occurred in the summer, however, the litterfall would have been comparatively dry and it seems likely that much/all of it probably burned in the fire.

These conjectures are supported by Cilliers et al. (2004) who reported reduced numbers geophytes and tussocks under burnt standing or stacked aliens burnt by wildfire. They went on to state "Buried indigenous propagules were largely destroyed by exceedingly hot fires in dense stands of living aliens and completely eliminated where stacks of alien slash were burnt by wildfires. The latent indigenous soil seed bank potential may therefore only be realised if alien biomass is removed from the area before hot summer burns occur."

Another obscuring factor in the Zachariashoek fire is that it is possible for some regions of the valley to have suffered great heat (high fire intensity and high fire severity) while others experienced moderate or low heat (low fire intensity and low fire severity - possibly even lower than the fire in the fynbos matrix). This may have been because an individual pine forest fire can have varying fire intensities and severities in the burn area as a result of microclimate variations in moisture, wind, and air-flow in the burn area (Neary *et al.* 2005).

Because of the synergy of these various factors, it is difficult to determine without further study the degree to which Zachariashoek fire was hotter in the pine afforested area, the spatial spread of such hotter fires throughout the valley, and/or the amount of heat conveyed to the original fynbos soil in the afforested sections of the valley. Such studies are beyond the scope of this project. Nevertheless, the broader evaluation, given that fires in invaded areas are hotter due to higher fuel bulk (Brooks *et al.* 2004), and that the heat of fires in alien pine stands does kill much of the indigenous seedbank surviving under the pines (Cilliers *et al.* 2004) is that the Zachariashoek fire was probably significantly hotter than surrounding fynbos fires and deliberate winter-season pine fires in most of the five research sites of this study.

*Methodological caveats*

The sampling methodology favoured the more common plant species and tended to underestimate locally rare species (Stohlgren 2007). Additionally, sampling plant species by area and volume (as opposed to absence or presence based purely on which plants emerge from the soil within the quadrat) means that vegetation with more spreading epigaeic parts (e.g. larger bushes) are favoured over smaller herbs in recording of absences or presences. In essence, sampling with species present in a volume over a quadrat means the "effective sampling area" for bushes and small trees is larger than the actual quadrat's ground area whereas for smaller herbaceous plants and smaller bushes the "effective sampling area" is closer to the

quadrat's actual ground coverage. However, any such biases would occur in both the Natural and PPR sub-sites as this was a comparative study so statistical differences are still valid.

The majority of the locally rare species (singletons, doubletons, and tripletons) were in the Natural sub-sites although a few were in the PPR sub-sites. Their generally higher presence in the Natural sub-sites than in the PPR sub-sites indicates that they may (generally) be slow at spreading, cannot compete with more aggressive plants re-establishing in the PPR sub-sites, or have specific micro-environment requirements which are not yet available in the PPR sub-sites. Perhaps after fifty years with a normal fire frequency, recovery of these locally rare species might be significantly improved.

Although some species had to be pooled due to field identification discrepancies, such pooled data are still in general agreement with genera for species that were easy to recognize consistently and as such could be pooled for inferences for the genus en bloc (Table 2.5 (b)). Particular care, however, is suggested in making conclusions for larger plant genera (*Searsia*, *Leucadendron*, and *Protea*) due to quadrat - plant size methodological problems.

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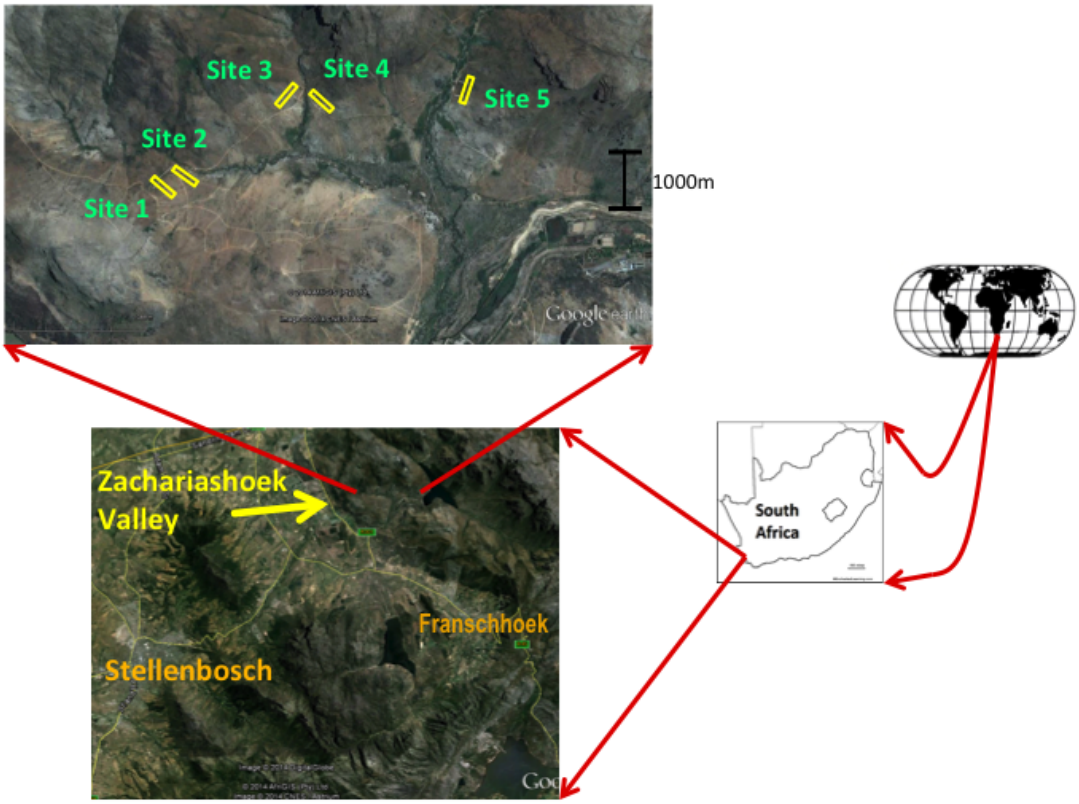



Figure 2. 1 Location of Zachariashoek Valley, Western Cape, South Africa, and of the five chosen replicate sites therein.

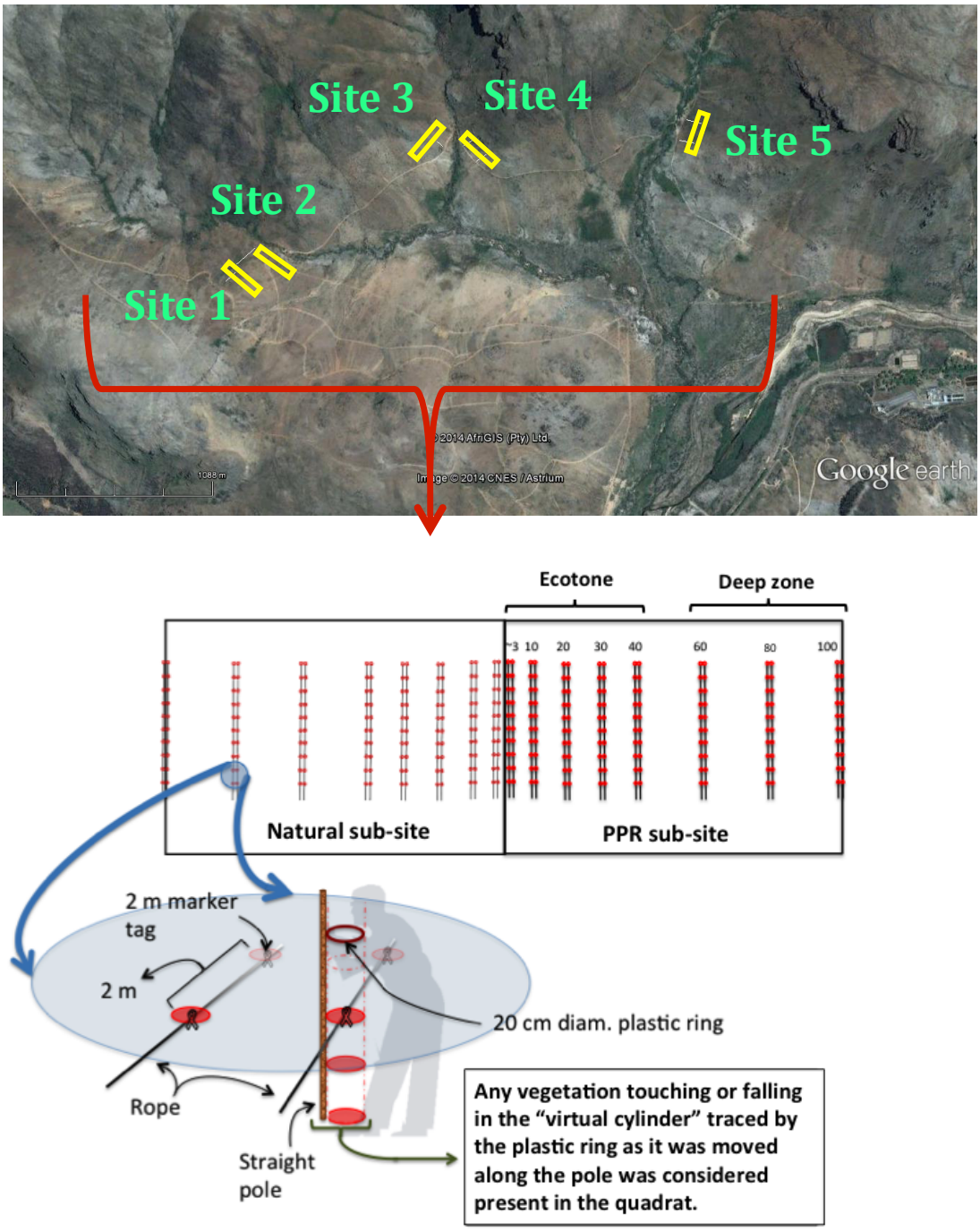



Figure 2. 2 Top: Google ™ image of study area. Center: schematic diagram of a single site showing the eight paired transects in each of Natural and Post-Pine Recovery sub-sites at 3 m, 10 m, 30 m, 40 m, 60 m, 80 m, and 100 m from the boundary. Bottom: Diagram of sampling method.

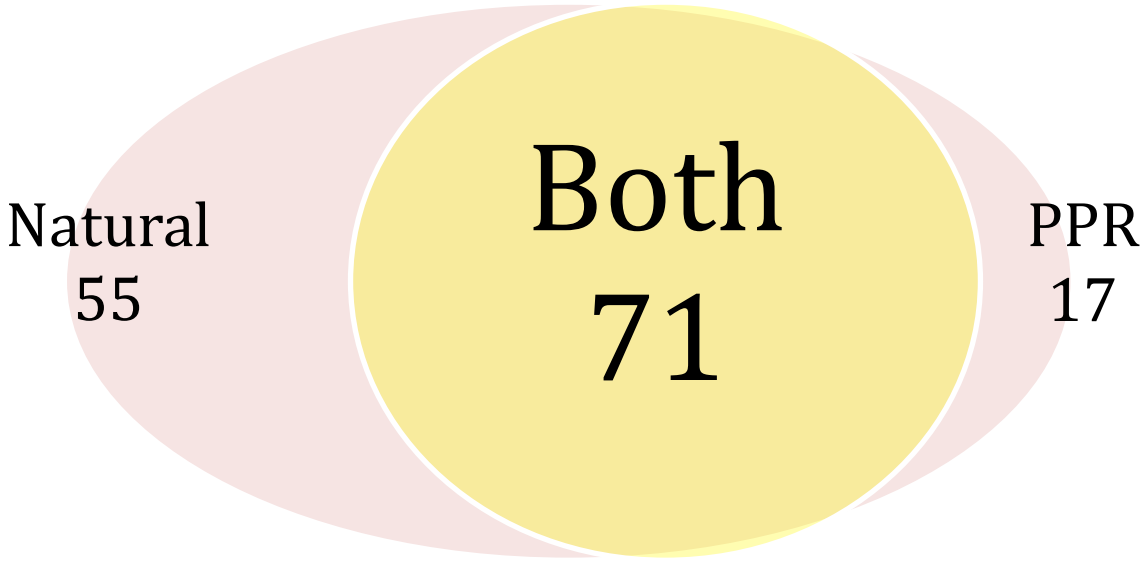



Figure 2. 3 Venn diagram of species unique to and shared between Natural and Post-Pine Recovery sub-sites.

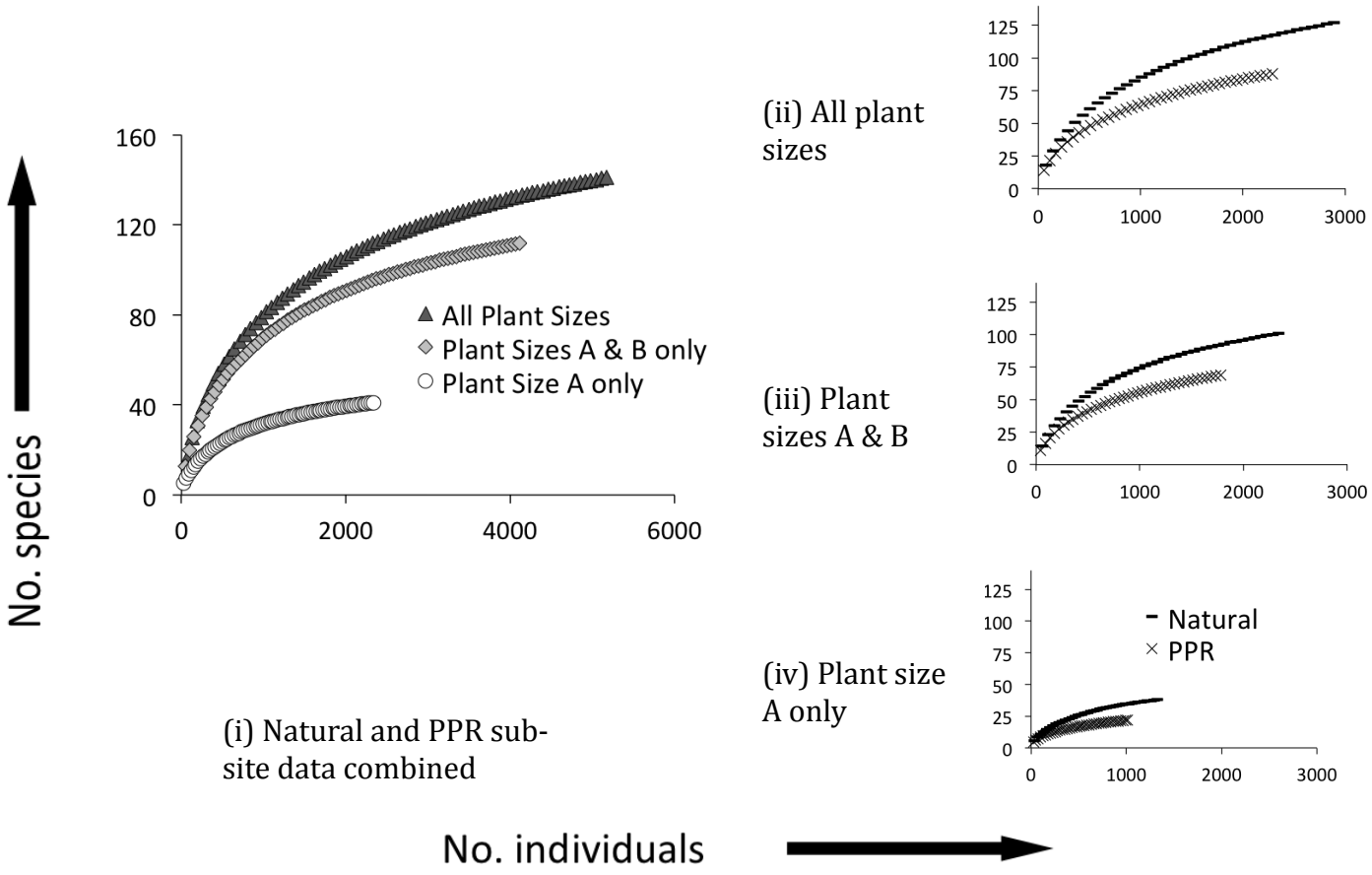
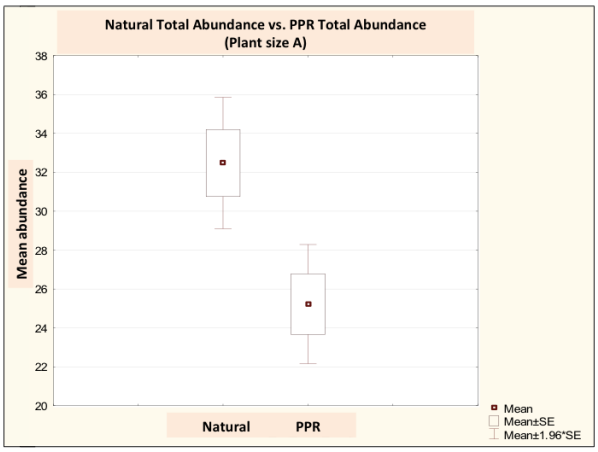
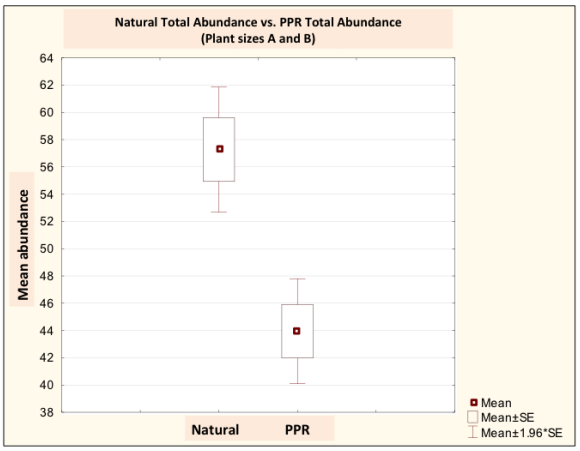



Figure 2. 4 Species accumulation curves for (i): plant sizes A and B combined with data for all plants in Natural and Post-Pine Recovery (PPR) sub-sites combined, (ii) all plant sizes in Natural and PPR sub-sites separately, (iii) plant sizes A and B in the Natural and PPR sub-sites, and (iv): plant size A only in the Natural and PPR sub-sites.




(i)

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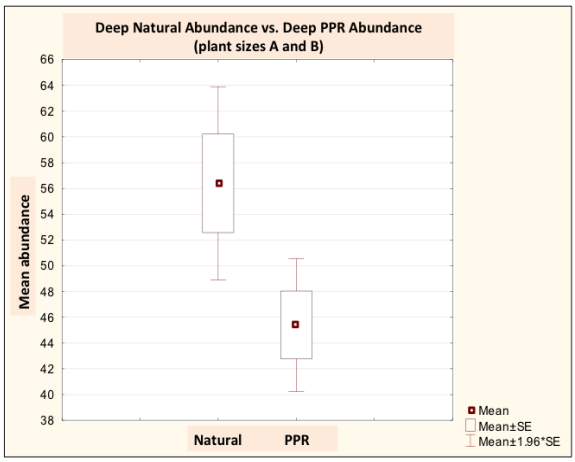


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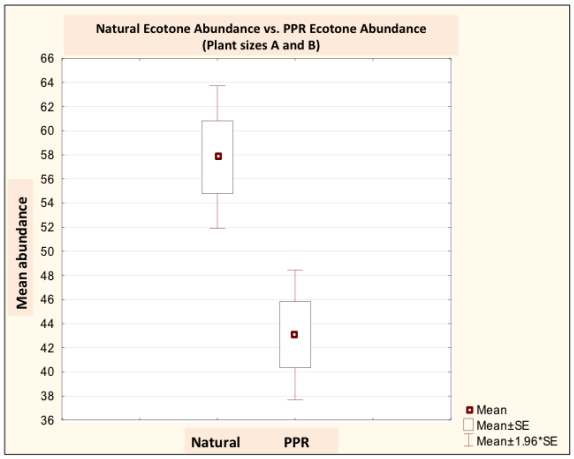
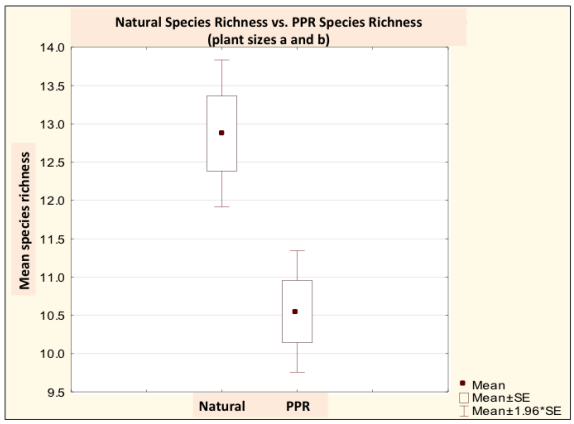
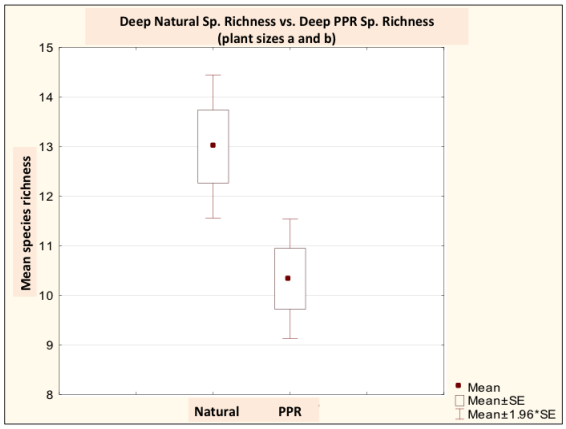



(iv)

Figure 2. 5 Paired box-plots comparing species abundance in Natural and Post-Pine Recovery sub-sites for size group A plants only (i), size group A and B plants together (ii), in Deep zones only (>50 m from the boundary) for size groups A and B plants together (iii), and for Ecotone zones only (<50 m from the boundary) for size groups A and B plants together (iv). (i) and (ii).




(i)

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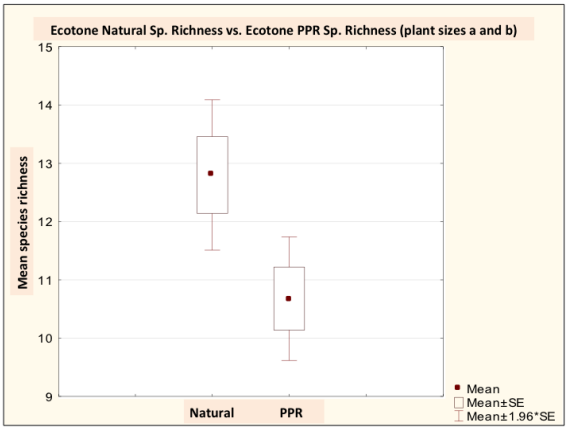



(iii)

Figure 2. 6 Comparison of species richness for plant sizes A and B combined for (i) Natural sub-sites vs. Post-Pine Recovery (PPR) sub-sites, (ii) Deep Natural sub-sites vs. Deep PPR sub-sites, and (iii) Natural Ecotone vs. PPR Ecotone.

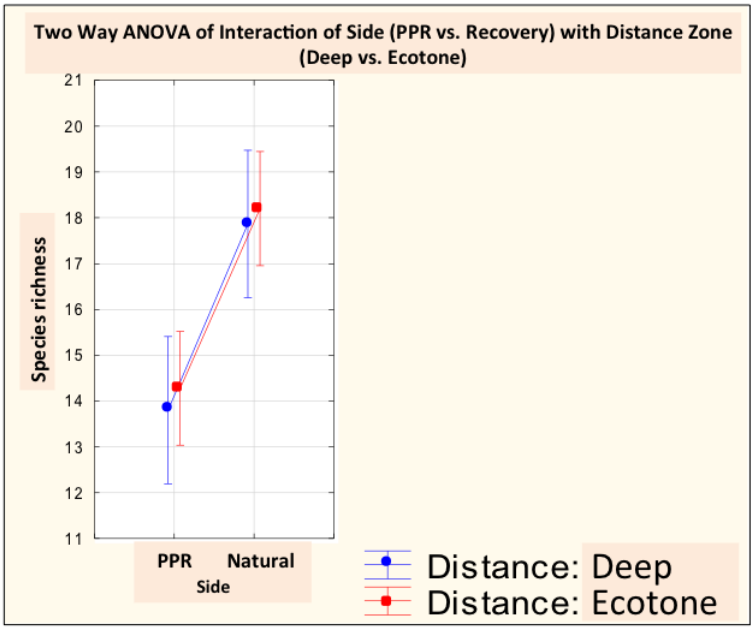
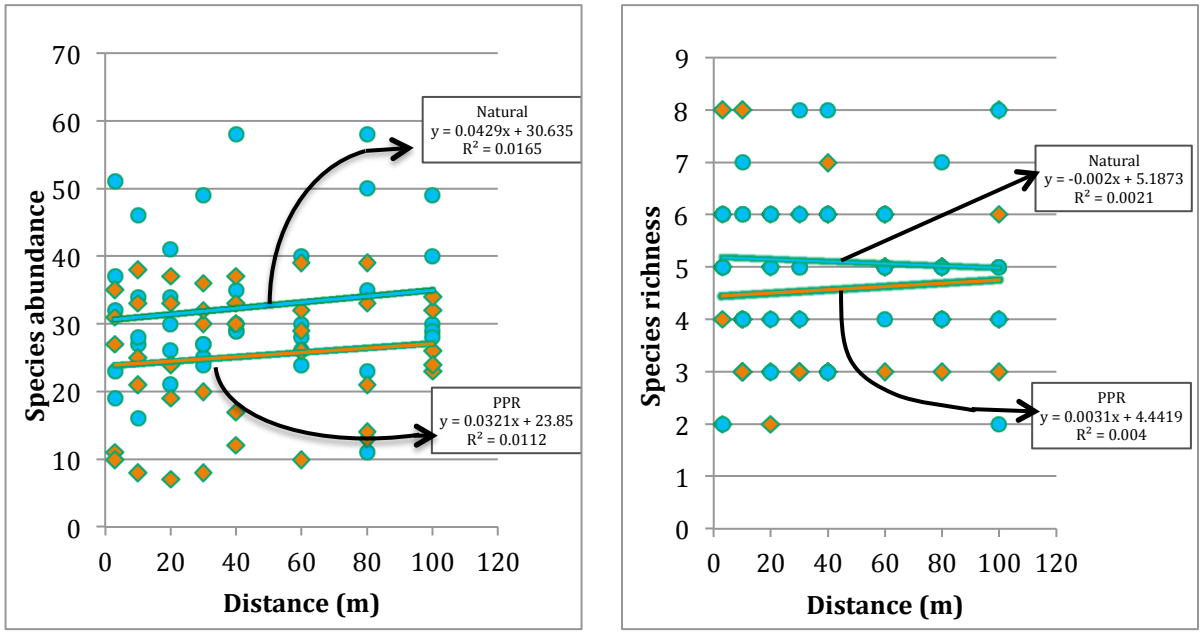
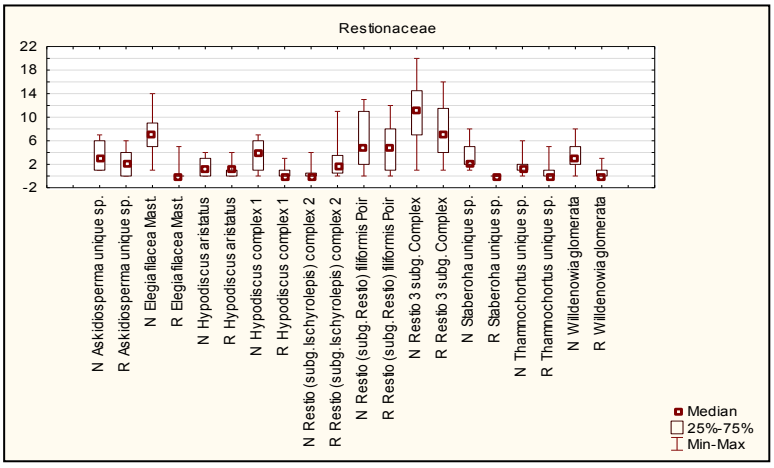



Figure 2. 7 Comparison of Species richness of Natural vs. Post-Pine Recovery sub-sites for Ecotone (red) and Deep (blue).

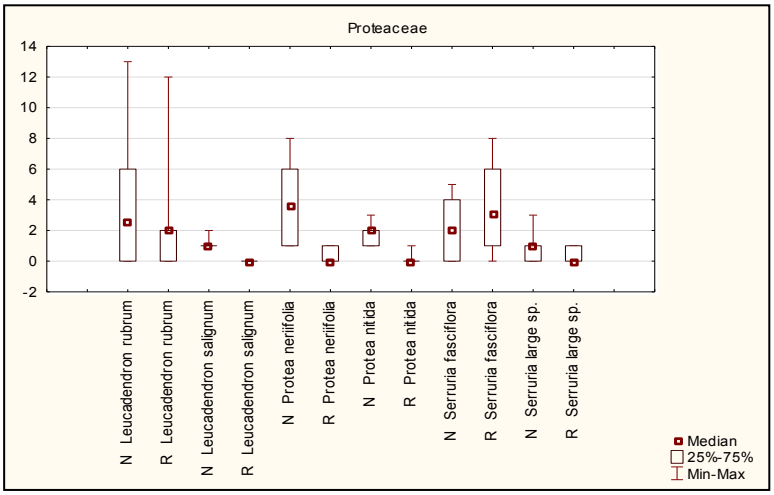



(i) (ii)

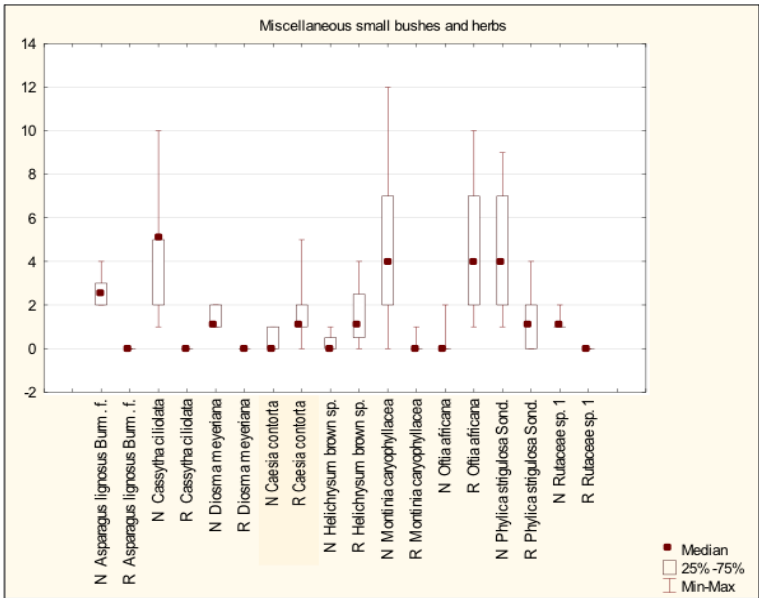
Figure 2. 8 Linear regressions of abundance with distance (i) and of species richness with distance (ii) for size A plants.




(i)




(ii)




(iii)

Figure 2. 9 (i) Restionaceae; (ii) Proteaceae; and (iii) Miscellaneous smaller bushes and herbs. Much of the Natural sub-sites' higher plant richness was characterized by individual species' complete absence in Post-Pine Recovery (PPR) sub-sites (N-Natural; R – PPR).

Table 2. 1 Geographical attributes of the five replicated sites used in this study. Each of the these sites was composed of two adjacent sub-sites, one a Natural sub-site (naturally recovering fynbos) and the other a Post-Pine Recovery sub-site.

| Site number | Coordinates | Elevation | Aspect | Slope |
|---|---|---|---|---|
| 1 | 33°49'40.0"S 19°02'04.9"E | 320 m | North-east facing (35° E of N) | ~100% (~45°) |
| 2 | 33°49'45.1"S 19°02'02.5"E | 405 m | North-east facing (35° E of N) | ~90% (~41°) |
| 3 | 33°49'25.5"S 19°02'42.2"E | 293 m | West – facing | ~30% (~14°) |
| 4 | 33°49'25.8"S 19°02'52.1"E | 310 m | SSW - facing (25° W of S) | ~75% (~34°) |
| 5 | 33°49'21.7"S 19°03'33.3"E | 290 m | West – facing | ~65% (~29°) |

Table 2. 2 List of all complexes of plants that were grouped as plant species complexes for identification consistency.

| Complex no. | Complex name | No. spp in complex | Species' names within complex |
|---|---|---|---|
| 1 | Pteridaceae spp. complex 1 | 2 | Pteridaceae sp. 1 |
| | | | Pteridaceae sp. 2 |
| 2 | Asteraceae spp. complex 2 | 3 | *Ursinia paleacea* (L.) Moench |
| | | | *Euryops abrotanifolius* (L.) Moench |
| | | | *Senecio burchellii* DC |
| 3 | Asteraceae spp. complex 1 | 2 | *Syncarpha canescens* (L.) B. Nord |
| | | | *Helichrysum zeyheri* Less. |
| 4 | Cyperaceae spp. complex | 15 | *Ficinia bulbosa* (L.) Nees |
| | | | *Ficinia duesta* (P. J. Bergius) Levyns |
| | | | *Ficinia ecklonea* (Steud.) Nees |
| | | | *Ficinia indica* (Lam.) Pfeiffer |
| | | | *Ficinia levynsiae* T. H. Arnold & Gordon-Gray |
| | | | *Ficinia nigrescens* (Schrad.) J. Raynal |
| | | | *Ficinia oligantha* (Steud.) J. Raynal |
| | | | *Ficinia tristachya* (Rottb.) Nees |
| | | | *Tetraria cuspidata* (Rottb.) C. B. Clarke |
| | | | *Tetraria nigrovaginata* (Nees) C.B. Clarke |
| | | | *Tetraria picta* (Boeck.) C. B. Clarke |
| | | | *Tetraria pubescens* Schonl & Turril |
| | | | *Tetraria sylvatica* (Nees) C. B. Clarke |
| | | | *Tetraria ustulata* (L.) C. B. Clarke |
| | | | *Tetraria variabilis* Levyns |
| 5 | *Anthospermum* spp. complex 1 | 2 | *Anthospermum aethiopicum* (L.) |
| | | | *Anthospermum capense* (L.) |
| 6 | *Hypodiscus* spp. complex 1 | 2 | *Hypodiscus striatus* (Kunth.) Mast. |
| | | | *Hypodiscus willdenowia* (Nees) Mast. |

Table 2.2 (Continued)

| Complex no. | Complex name | No. spp in complex | Species' names within complex |
|---|---|---|---|
| 7 | Poaceae spp. complex | 11 | *Ehrharta calycina* Sm. |
| | | | *Ehrharta longifolia* Schrad. |
| | | | *Ehrharta ramosa* (Thunb.) Thunb. |
| | | | *Geochloa* (*Merxmuellera*) *rufa* (Nees) Conert |
| | | | *Pentameris* (*Pentaschistis*) *curvifolia* (Schrad.) Stapf. |
| | | | *Pentameris* (*Pentaschistis*) *eriostoma* (Nees) Stapf. |
| | | | *Pentameris* (*Pentaschistis*) *malouinensis* (Steud.) Clayton |
| | | | *Pentameris* (*Pentaschistis*) *pallida* (Thunb.) H. P. Linder |
| | | | *Themeda triandra* Forssk. |
| | | | *Tribolium uniolae* (L.f.) Renvoize |
| | | | ? *Tenaxia* (*Merxmuellera*) *stricta* (Schrad.) Conert |
| 8 | *Restio (*subg. *Ischyrolepis)* spp. complex 1 | 2 | *Restio* (subg. *Ischyrolepis*) sp. 1 |
| | | | *Restio* (subg. *Ischyrolepis*) sp. 2 |
| 9 | *Restio (*subg. *Ischyrolepis)* spp. complex 2 | 2 | *Restio* (subg. *Ischyrolepis*) *sieberi* Kunth. |
| | | | *Restio* (subg. *Ischyrolepis*) *monanthus* Manth. |
| 10 | *Restio* spp. complex 3 | 4 | *Restio* (subg. *Ischyrolepis*) *capensis* (L.) H. R. Linder & C. R. Handy |
| | | | *Restio* (subg. *Ischyrolepis*) *gaudichaudiana* |
| | | | *Restio* (subg. *Locapsis*) sp. |
| | | | *Restio* (subg. *Restio*) sp. |

Table 2. 3 Visual observations of: (i) obvious soil disturbances, (ii) vibrantly larger/lusher plant species in Post-Pine Recovery (PPR) sub-sites, and (iii) larger plant species noticeably absent (close to absent) from PPR sub-sites. These are "general quick observations" that come across to the observer from a simple glance.

| **Site** | **(i) General observations on level of soil disturbance** | **(ii) PPR sub-site's noticeably lush/vibrant plant species** | **(iii) Natural sub-site's larger plant species absent or nearly so in PPR sub-site** |
|---|---|---|---|
| 1 | Significantly more and looser soil in PPR sub-site; significant rill erosion (donga) in the PPR sub-site; Natural sub-site soil firmer; | *Metalasia densa*, *Stoebe plumosa*, *Stoebe cinerea* | Larger *Protea* spp. |
| 2 | Significantly more and looser soil in PPR sub-site; significant rill erosion (donga) in the PPR sub-site; Natural sub-site soil firmer; | *Metalasia densa*, *Stoebe plumosa*, *Stoebe cinerea* | None noticeable |
| 3 | Significantly looser soil on PPR sub-site; Natural sub-site soil was firmer | *Stoebe plumosa*, *Stoebe cinerea* | Larger *Protea* spp. |
| 4 | Significantly looser soil on PPR sub-site; Natural sub-site soil was firmer | *Stoebe plumosa*, *Stoebe cinerea* | Larger *Leucadendron* spp. |
| 5 | Similar appearance on both Natural and PPR sub-sites | *Stoebe plumosa*, *Anthospermum* spp. | Larger *Protea* spp. |

Table 2. 4 Individual species comparison of abundance between Natural vs. Post-Pine Recovery (PPR) sites. Values are number of species which met the criteria in a Wilcoxon's matched pairs test. Only species with over 6 occurrences were included in this analysis (n=50). Half had significant differences in abundance between PPR and Natural sub-sites, with a tendency to be higher at the Natural sub-sites. Data within square brackets indicate possible maximum number of species due to presence of plant species complexes.

| **Grouping** | **No significant difference** | **Natural sub-sites were higher** | **PPR sub-sites were higher** |
|---|---|---|---|
| Restionaceae | 5 [6] | 2 [3] | 1 [4] |
| Proteaceae | 3 | 3 | 0 |
| Herbs & small bushes | 16 | 10 | 5 [7] |
| Total | 24 [25] | 15 [16] | 6 [11] |

| | | | | |
|---|---|---|---|---|
| Total spp. # for which a difference was seen (whether higher in N or higher in P) | = | $\frac{15+16}{2}$ | + | $\frac{6+11}{2}$ |
| 24 (±3) | = | 15.5 | + | 8.5 |

(lower limit = 15 + 6 = 21)
(upper limit = 16 + 11 = 27)

Table 2. 5 Natural and Post-Pine Recovery (PPR) sub-site abundance data, Wilcoxon's T statistics, and associated P-values for plant complexes (a) and for plant genera (b). Significant P-values are in **bold**. The side with the higher level is indicated in square brackets [ ].

(a)

| Complex Name | Natural Abundance* | PPR Abundance* | N | Number sp. in complex | Wilcoxon's T | P-value |
|---|---|---|---|---|---|---|
| Asteraceae spp. complex 2 | 1.6±2 [1] | 2.6±2 [2] | 30 | 3 | 132.5 | **0.040 [PPR]** |
| Cyperaceae spp. complex | 4.8±3.7 [4] | 5±3.7 [5] | 33 | 15 | 267.5 | 0.816 |
| *Anthospermum* spp. complex | 4.2±3.6 [4] | 3.5±4.2 [2] | 36 | 2 | 276 | 0.371 |
| *Hypodiscus* spp. complex 1 | 3.7±2.4 [4] | 0.5±1 [0] | 11 | 2 | 4 | **0.010 [N]** |
| Poaceae spp. complex | 11±5.3 [11.5] | 9±4.4 [9] | 37 | 11 | 223.5 | 0.053 |
| *Restio* (subg. *Ischyrolepis*) spp. complex 2 | 0.5±1.2 [0] | 2.6±3.1 [1.5] | 12 | 2 | 15.5 | 0.065 |
| *Restio* spp. complex 3 | 10.8±5.1 [11] | 7.3±4.4 [7] | 38 | 4 | 143 | **0.001 [N]** |

(b)

| Genus | Natural Abundance* | PPR Abundance* | N | Number sp. in complex | Wilcoxon's T | P-value |
|---|---|---|---|---|---|---|
| *Aspalathus* spp. | 0.8±0.9 [1] | 1.8±2.2 [1] | 16 | 4 | 1.112 | 0.266 |
| *Erica* spp. | 2.3±1.6 [2] | 1±1.3 [0.5] | 24 | 7 | 3.000 | **0.003 [N]** |
| *Helichrysum* spp. | 0.5±0.7 [0] | 1.4±1.2 [1] | 18 | 6 | 2.090 | **0.037 [PPR]** |
| *Hypodiscus* spp. | 2.6±2.6 [3] | 1±1.1 [1] | 23 | 3 | 2.388 | **0.017 [N]** |
| *Leucadendron* spp. | 3.9±3.9 [3] | 2.2±3.2 [2] | 22 | 3 | 1.380 | 0.168 |
| *Protea* spp. | 2.8±2.3 [2] | 0.2±0.4 [0] | 19 | 6 | 3.823 | **<0.001 [N]** |
| *Asparagus* spp. | 2.4±1.4 [2] | 1±1.5 [0] | 6 | 3 | 1.468 | 0.142 |
| *Restio* spp. | 13.7±8.2 [12] | 10.1±7 [8.5] | 38 | 8 | 2.705 | **0.007 [N]** |
| *Searsia* spp. | 1.4±2.1 [1] | 0.7±1 [0] | 7 | 5 | 0.338 | 0.735 |

* Mean, ± standard deviation, [median]

# Chapter 3 – Overcoming the Observer Effect when sampling flower-visiting insects


## Abstract

Sweep netting is a common methodology for sampling insects on and in vegetation, mostly by dislodgement. However, along with pan traps, sweep netting is also used for collecting flower-visiting insects (anthophiles) such as certain bees and beetles. In the case of flying insects at flowers, the movement of the human sweep netter is a factor that can alert insects into escape behaviour. This means that sampling may not be representative of natural population levels of the anthophiles. This effect of human activity on the focal anthophilic insects is called here 'The Observer Effect'. It is distinguished from inter-observer/collector variability, referring to differing skill levels/catching techniques between different observers in seeing or catching insects. I performed a test of the effect of the movement of an observer by observing bees over 2-6 min and over 10-13 min waiting periods. Overall, significantly more bees per minute per ln(no. flower umbels) were observed during longer observation periods. This indicates that bees move away from the movement of the observer. They then slowly return in increasingly greater numbers with longer waiting periods. A proposed protocol for efficiently employing these different methods for sampling anthophiles is to walk to the observation plot, set up the pan trap, make observations while standing still, sweep net for bees and then to move to the next observation plot.


## Introduction

Sweep netting is a traditional dislodgement method of catching and sampling insects for research with variations and permutations of this method being many. Among these variations is to capture flying insects as they hover close to or land on flowers. Typically, transects are walked, during which collectable insects are caught as the net is swept back and forth through and above the vegetation (Samways *et al.* 2010). The human sweep netter or observer may or may not consciously try to catch or focus on catching a specific family, genus, or even species of insect depending on the specific goal of the study.

Briefly, the Observer Effect (OE) (as used in this chapter) is distinguished from observer/sweep-netter bias. In the case of the OE, the mere presence and movement of the observer affects the behaviour of the insects irrespective of the identity of the observer, while the observer/sweep-netter bias refers to innate inter-observer differences in abilities, skills and techniques of observing/catching the insects.

The OE has been discussed (Baker & McGuffin 2007, Basset *et al.* 2012, Campbell *et al.* 2012, Pontin *et al.* 2006, Wade *et al.* 2005) but the extent of this effect in Cape Floristic Region (South Africa) sclerophyllous, flower-rich fynbos has not been investigated, even though sweep netting is a commonly used

method. This motivates the question: How much error is inherently present in sweep net data from the OE? At least some estimate of error is necessary for good data confidence. Also, mitigation measures of the OE have included waiting periods of one minute (Pontin *et al.* 2006) to allow for habituation of insects to the presence of the observer. A second question arises here: Is this sufficient time, or will waiting longer periods entice even larger numbers of insect flower visitors?

The aim of this chapter is to determine whether the number of bee visitations per minute per umbel on a specific mass-blooming flowering bush, *Metalasia densa* (Lam.) P. O. Karis (Asteraceae), a suitable focal plant for this study in view of its bee attractiveness, changes with increased time after initial observer disturbance.

**Materials and Methods**

This study was conducted as part of a larger study (Chapters 2 and 4) in the Zachariashoek Valley (33.8291º S, 19.0518º E), Hawequas Nature Reserve (part of the larger Limietberg Nature Reserve), Western Cape, South Africa (Figure 3.1). This area was partially afforested with a managed pine plantation and was partially maintained as a pine-free, nature reserve. The entire area, pine trees and natural vegetation (fynbos), burned in 1999. After the fire, the afforested area was not replanted with pines and the whole area was managed as a nature reserve with regenerating pines actively removed.

(Figure 3.1)

*Metalasia densa* (Lam.) P. O. Karis (Asteraceae) was chosen for this study as it attracts a large variety of generalist species, many of them bees of which most are important pollinators in the ecosystem. This was preferable to a specialist pollination syndrome plant which would provide results with limited applicability to other taxa. *Metalasia* spp., locally known as honeybush ('heuningbos'), has an intense honey-like smell when in full bloom. There is evidence that certain insects' attraction to various flowers or food sources is dependent or otherwise influenced by the size of the food source such as number of flowers (Westphal *et al.* 2003). The flower of *M. densa* is typically light beige and forms umbels which have pollenaria that extend out of the flower when in bloom and this (and possibly any nectar in extremely small nectaries within the flower) is highly attractive to many anthophiles (Figure 3.2). In the 13-year old post-burn fynbos site here, the plant varied in size, reaching heights of up to 200 cm and diameters up to 300 cm, with typical bushes being 50 to 150 cm tall and 50 to 200 cm wide.

(Figure 3.2)

The data extracted for this chapter are actually part of a larger sweep net study which will be discussed in Chapter 4. In the sweep net study, the main aim was to catch bees in different study areas and

sites for identification and analysis of bee species abundance and richness. In the process of sweep netting I would stop at blooming *M. densa* bushes and wait for bees and then attempt to catch them with a sweep net when the opportunity arose. Often however, due to short flower visitation time and/or the position of bees on the bush, there was no opportunity to catch them. Due to this possibility I would always count bees and tabulate these count data (distinguishing between honeybees and non-*Apis* bees) to allow for statistical analysis of such strictly *observation* sessions separately from actual sweep netting data. To this end, failed sweep netting attempts were duly noted in order to exclude sweep-netting disturbed sessions and make the only disturbance my approach to the bush before observation (the "control" disturbance). A total of 57 such observation periods were used to evaluate the effect of time on number of bee visitations observed after my initial observer disturbance of approaching the bush.

Each observation period was at a different bush and these observation periods were performed between 9:00 hr and 18:00 hr on four minimally cloudy or cloud-free (<20% cloud coverage), calm (wind speed < 7 km/h) days, from 29 March 2010 to 19 May 2010. After approaching the bush, I stopped, stood still and counted the number of bee visitations for a period of up to 13 min (see below). My observation times were split into two categories: short (2-6 min; n=21) or long (10-13 min; n=36). The longer of these periods was chosen to be substantially longer than what other researchers have used as a waiting period for approach disturbance to calm down (e.g. 1 minute by Pontin *et al.* 2006) while still being short enough to allow focussed, motionless counting without errors due to fatigue. Preliminary observations suggested that 13 min should be the maximum. I chose this other shorter time period of 3-6 min, arbitrarily, to be about half or less than half my maximum waiting period of 10-13 min. All sessions were concluded with a count of the *M. densa* umbels on the observed bush (or section of observed bush if it was large) that had pollenaria protruding from the umbel flowers and available for pollen-foraging bees (Figure 3.2 (iii)).

**Statistical analysis**

Due to differences in observation sessions in the number of umbels at a given observation area, and due to the differing time periods for observations, bee visitation was transformed into number of visitations per min. However, as the number of pollen-providing umbels in observation plots differed much and was highly skewed, these data were further transformed to ln(no. umbels) and then the number of bee visitations per min per ln(no. umbels) was calculated. A linear regression was performed with observation time as the independent variable and number of bee visitations per min per ln(no. umbels) as the dependent variable. All analyses were performed using R (version 3.1.1, R development core team 2014).

**Results**

There was a significant relationship between the number of minutes elapsed post-disturbance with number of bee visitations per min per ln(no. of umbels) (Slope = 0.0075, Standard Error of the slope = 0.0034, $R^2 = 0.080$, t = 2.19, P = 0.033) (Figure 3.3).

(Figure 3.3)

**Discussion, recommendations, and conclusion**

The presence of a clear OE is shown here, and its implication for bee research is that, for observation plots, wait times of at least five or, even better, 10 min periods should be used. Figure 3.3 shows that with increased observation time, more bees are observed per min per ln(no. of umbels). This result strongly suggests that generalized transect sweep netting (walking along a transect while sweeping the net back and forth without aiming for any one insect) is significantly less effective for obtaining a representative sample of bees as (potentially many) more observer-wary bees will not be anywhere in the locality of the sweep net during such disturbance. To address this deficiency, an alternative protocol should be instigated, which would combine observation plots with sweeping by observing first for a 5-10 min period and recording results on a chart or audio recording, and then trying to sweep net the bees that are being researched with insect-specific "ambush" sweep-netting. Furthermore, such observations should be done by one person with assistants and other observers remaining at a significant distance (e.g. >20 m away). These measures would mitigate the OE. However, to sidestep it completely would require the added use of unattended pan traps, flight intercept traps, or a recently developed pan and flight intercept combination (PAFIC) trap (see Chapter 5). Ideally, observation plots, sweep netting, and passive trapping methods should be used in combination, as there are likely to be some bee species that are caught by one method but not others (Samways *et al.* 2010). An efficient protocol for employing these different methods would be to walk to the observation plot, set up the pan trap, make observations, sweep net for bees and then to move to the next observation plot. This method should successfully lead to more reliable data for bees and possibly also other focal insect taxa. This approach is also likely to have applicability to other regions of the world as well.

This research was focussed on flower-visiting bees in the fynbos. The degree to which it is applicable to other anthophilous insect groups (wasps, flies, bugs, beetles) is arguable though a significant portion of them likely exhibit at least some observer-shy behaviour. Of course, some pollinators such as sweat bees (within the Halictidae and Apidae) and some blood-meal seeking but also anthophilous flies (e.g. Diptera: Tabanidae) would presumably exhibit observer-attraction behaviour, so they are not the focal taxa of this discussion.

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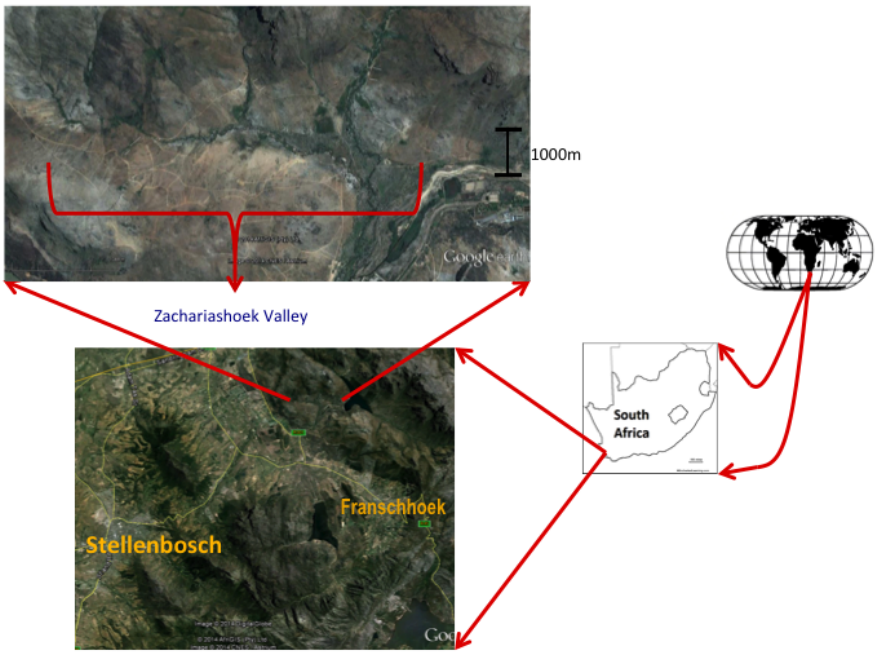


Figure 3. 1 Location of Zachariashoek Valley.

(i) (ii) (iii)

Figure 3. 2 (i) *Metalasia densa* blooming phenology: flowers on left are in later stages and flowers on right in earlier/mid stages. (ii) *Apis mellifera* active on *M. densa*. (iii) Slightly lighter coloured *M. densa* flower (notice protruding yellow pollenaria indicating flower is blooming and suitable for pollen foraging insects). Photos (i) and (iii), respectively, with kind permissions of Micky Orrey and Grant Alexander.

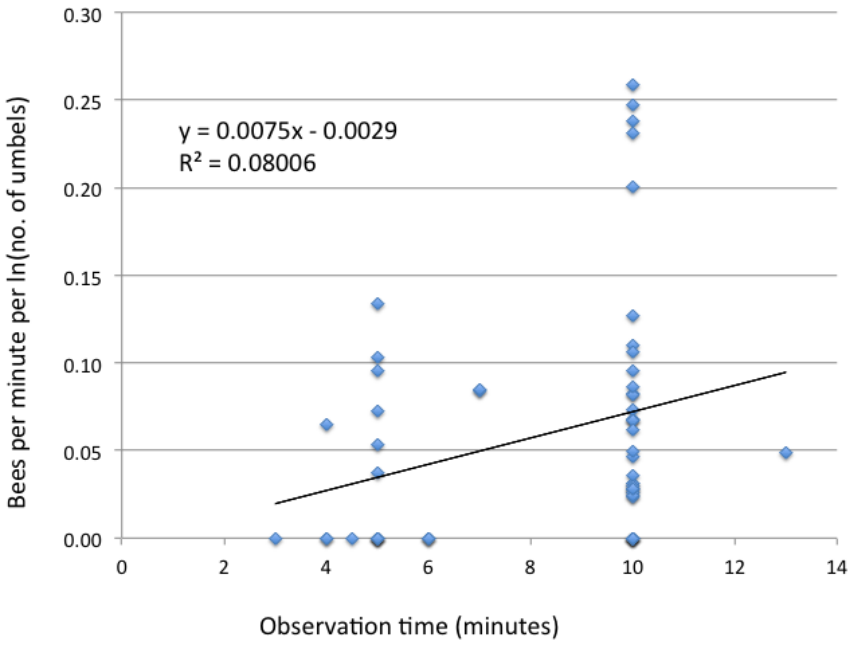


Figure 3. 3 Regression analysis of observed bees per minute per ln(no. of umbels) vs. observation time (minutes) (t = 2.19, P = 0.033).

# Chapter 4 – Effect of pine afforestation disturbance on bee recovery after fire in the Cape Floristic Region biodiversity hotspot


## Abstract

Successional recovery of vegetation after a disturbance such as afforestation with alien invasive trees is necessarily linked to presence and diversity of insect pollinators. However, little is known about the effect of invasive alien tree afforestation on the recovery potential of pollinator assemblage areas after the removal of such timber stands. Here, I test whether diversity of bees, the most common insect pollinators, is correlated in the same post-fire successional stage of two adjacent ecosystems: one with a history of Monterey pine (*Pinus radiata* D. Don) afforestation and one without this disturbance. In 1999, a fire burned both the pine forest and surrounding natural fynbos; both sites were left to naturally recover after the fire with no intervention except reclearing of pine tree regrowth. I tested seasonal and spatial aspects of bee assemblage recovery in a series of sampling sessions in five replicated sites in the Zachariashoek valley in Western Cape montane fynbos. I employed Individual Bee Sweep (IBS) net sampling and a modified Pan and Flight Intercept Combination (PAFIC) trap sampling at the ecotone between formerly pine-covered fynbos (Post-Pine Afforestation/Pine Forest Fire Recovery (Post-Pine Recovery for short: 'PPR') and the natural ('Natural') fynbos ('Ecotone') and and at various distances away from the ecotone ('Deep' zones). Traps were placed in paired Natural and PPR sub-sites that had the same topography, elevation, and aspect. IBS net sampling was conducted in autumn focusing on one mass flowering bush, *Metalasia densa*, and in spring on a variety of flowers. In the springtime, PAFIC traps were placed adjacent to a mass-flowering bush, *Agathosma capensis*. In early summertime, PAFIC traps were placed in the centroid of flower concentrations which were somewhat sparse (compared to the spring sampling). Both Natural Ecotones and Natural Deep zones had higher bee species abundance than their counter-part PPR zones. In spring and early summer (PAFIC) sampling, there was no significant difference in bee species richness between Natural and PPR sub-sites (Spring sampling: Natural - 20 species, PPR – 20 species; Early summer sampling: Natural – 20 species, PPR – 21 species). A combination of soil disturbance and concomitant disturbed plant succession caused by the pines and the hotter (possibly much hotter) nature of the 1999 pine forest fire is indicated as the most probable reason for lower bee counts in PPR sub-sites. A remarkably high total of 56 species of bees were recorded for the one valley under research from the methods employed in this study. This number rivals that of other centers of high apifaunal biodiversity in the world suggesting that the Cape Floristic Region may have the highest bee biodiversity in the world. Of these, some 37 to 44 are undescribed to science.

## Introduction

*Insect biodiversity and its relevance to balance in ecosystem processes*

Much of the estimated US$ 16 to 54 trillion ($10^{12}$) per year value of biodiversity's ecosystem services (Costanza *et al.* 1998) is directly attributable to plant biodiversity. However, insect-plant interactions have been co-evolving for a long time (insect herbivory: 420 mya (Labandeira 2005), insect pollination: ~120 to 140 mya (Grimaldi 1999), general interactions: 97 mya (Labandeira *et al.* 1994)). This makes all such interactions essential for natural ecosystem functioning, with some, such as pollination, so important as to be called keystone processes. However, as the majority of species in natural terrestrial ecosystems are insects (Samways 2005), healthy, stable insect populations (within the parameters of normal variations), or the lack thereof, are definitive indicators of the healthy ecosystem processes that they are part of.

*Present environmental crisis involves a pollination crisis*

In the present environmental crisis, threats to the keystone process of pollination have been referred to as the pollinator crisis (Buchmann & Nabhan 1996a, Holden 2006, Tylianakis 2013, Wilcock & Neiland 2002). Declines in pollinators mirror declines in insect diversity in general (Mawdsley & Stork 1995, Samways 2005) and are coupled with threats to the biodiversity of naturally occurring plant species as well as agriculturally important crops (Garibaldi *et al.* 2011).

The breakdown of animal-mediated pollination is associated with decreases in all anthophile species from an estimated 300 000 flower visitors world-wide (Nabhan & Buchmann 1997). The majority of these species are insects in the super-speciose Hymenoptera, Coleoptera, and Diptera families. The pollinator decline has been severe in the case of one extremely important pollinator: the honeybee (*Apis mellifera* L. subspp.) (Aizen *et al.* 2009, Buchmann & Nabhan 1996b, Gill 1991, Tylianakis 2013). Some crops that are pollinated mainly by honeybees are also pollinated by non-honeybee pollinators and this can result in greater yields (Ramirez & Davenport 2013, Richards 1993). If decreases in honeybee populations extend to other bee species' populations and to other pollinators in general, then the picture, all premised under a simple causality, is a stark one: a breakdown in the pollination of the 90% of all flowering plants that require insect-mediated pollination. This would mean serious reproductive threat to these plants and a decrease in their healthy populations, especially wild populations, with possibilities of extinctions not long thereafter (Buchmann & Nabhan 1996a, Kearns *et al.* 1998).

There is some debate on how serious or real the pollination crisis really is (Ghazoul 2005). However, global warming, pollution, unnecessary or excessive use of pesticides, and, most harmful, habitat destruction and invasive species interact synergistically to threaten pollination in major ways. The reality is

that extinction resulting from pollination web breakdowns is a threat and the breakdown of pollination webs has been shown to occur (Pauw 2007). Evidence strongly suggests that loss of pollinator functional diversity can reduce crop yields, cause natural plant population declines, and even lead to extinctions in wild plant species (Buchmann & Nabhan 1996a, Fontaine *et al.* 2006, Kearns *et al.* 1998, Memmott *et al.* 2004).

A current critical question is whether pollinators and pollination can recover after decline through restoration of the landscape. While most restoration research has tracked the recovery of plant communities over time, there is an inherent feedback process which dictates that insect-pollinated plants cannot be restored to viable reproductive communities without their associated pollinators. Because pollinators are mobile and therefore may recover more easily than their plant hosts, few studies have specifically investigated pollinator recovery post-disturbance or during restoration processes. Such basic information on the recovery of pollinators is important given the present global pollination crisis. Information on this is required for making informed management decisions on, for example, restoration following removal of alien trees.

*Conservation focus: the Western Cape as a biodiversity Hotspot under threat from invasive plants*

The Western Cape of South Africa has within it one of the world's six floristic kingdoms, the Cape Floristic Region (CFR), characterized by evergreen, sclerophyllous heath and shrubland. The CFR is one of five global regions with a Mediterranean-type climate, having cool, wet winters and hot, dry summers. The plant diversity of the CFR represents some 20% of plant species in Africa concentrated in 0.5% of the continent area (UNESCO 2014) and is classified as a world biodiversity hotspot (Myers 1990, Myers *et al.* 2000).

The largest conservation threat to the CFR is habitat transformation due to invasive species, agriculture, and urban development (Gaertner *et al.* 2009, Rouget *et al.* 2003, Picker & Griffiths 2011). Of the invasive species, pine trees are particularly important as they are highly invasive in the southern hemisphere while also being cultivated for timber (Le Maitre 1998, Richardson 1998).

Managed pine forests in the CFR can shade out other vegetation, leading to localized extinction for many plants on the forest floor and for many insects (Figure 1.1). As a consequence, most normal insect-fynbos ecosystem processes also become functionally extinct and this includes animal mediated fynbos pollination.

*Fynbos' natural fire regime and the restoration/recovery of insects and fynbos after pine tree removal*

Naturally-occurring fire forms an integral part of fynbos (Allsopp *et al.* 2014, Bond *et al.* 2005), with an estimated natural burn frequency of about 15 to 20 years with significant variation from this depending on the specific area of the CFR (Southey 2009). In contrast to natural fires, fire in pine forests in the CFR burn hotter than fynbos, owing to the higher fuel load (Brooks *et al.* 2004). Soils that are subjected to such hot fires lose some capacity to absorb water (DeBano 2000, Scott & van Wyk 1992) and this may hamper the ability of many fynbos-associated insects and fynbos plants to re-colonize such areas.

*Caveat on restoration and recovery terminology for this thesis*

Restoration and recovery terms are sometimes used interchangeably. For clarity in this thesis, restoration will be taken to mean the efforts of restoration ecologists to return an ecosystem to some historical state (Falk *et al.* 2006). In the context of the research area under study here, no active restoration effort was performed (not infrequently an actual intended ecological restoration strategy) other than systematic clearing of the area from re-growth of invasive tree species. For this reason I use the term "recovery" as it more effectively implies the passive nature of maintenance in the Zachariashoek area. The term "restoration" will be contextualized as it is used to avoid confusion.

*Objectives*

Keeping the above in mind, there are two objectives of this chapter:

(1) To compare bee abundance and bee species richness among Natural fynbos and fynbos Post Pine Recovery (PPR) areas, both of which are in the same post-fire successional stage.

(2) To draw preliminary conclusions regarding observed differences.

**Materials and methods**

*Study sites*

The study area was located in the Zachariashoek Valley (33.8291 S, 19.0518 E) in the southwestern corner of the Limietberg Nature Reserve, Western Cape Province, South Africa. In the Zachariashoek valley a private forestry company (Mountains to Oceans Forestry (Pty) Ltd) had managed a Monterey pine (*Pinus*

*radiata* D. Don) plantation for 40 + years. This managed forest was in a natural fynbos matrix in what is now called the Limietberg Nature Reserve (formerly Haweqas Nature Reserve). This reserve was managed by the provincial conservation authority, CapeNature. Management of landscapes in the area involved removal of seedlings from propagules from the plantation that had established in the fynbos matrix. In 1999, an extensive fire swept throught the entire area burning down both the plantation and the surrounding fynbos matrix. The entire area was left to recover naturally after the fire with no intervention except reclearing of pine tree regrowth.

Five sites were selected each of which contained two adjacent, paired sub-sites which had been burned in the fire of 1999. The five sites were chosen so as to pair five natural, reference fynbos sub-sites ('Natural' sub-sites) with five post pine recovery sub-sites ('PPR' sub-sites). All sites were in the Haweqas Sandstone Fynbos biome (FFs 10) (Mucina & Rutherford 2006). Each of these pairs was adjacent i.e., there was a Natural sub-site and a PPR sub-site next to each other with the transition being referred to here as the Natural/PPR boundary (Figure 2.1); for photographs of the five sites see Appendix 4). All paired Natural and PPR sub-sites had the same elevation and topography. Grid references, elevation, aspect, and slope details of the sites are given in Table 2.1.

*Insect sampling*

Insect sampling was done in three initial Sampling Sessions (SS's) to examine appropriate sampling technique for a good sampling protocol and as such are mentioned first (SSs 1, 2, and 3) as they did yield some useful information. The statistical data analysis was however only anticipated from the bee sampling that I did in SSs 4, 5, 6, and 7 with the modified trap that I developed (see Chapter 5), the Pan and Flight Intercept Combination (PAFIC) trap (Figure 5.1).

Sweep netting was done in two Sampling Sessions (SS's): one (SS 1) in March to May (Autumn) 2010, and the other (SS 2) in October (Spring) 2010. The bee sampling was done with a standard sweep net with a diameter of 40 cm and a depth of 1 m, attached to a stout 1.5 m wooden pole.

Traditional pan trap sampling was initially done (SS 3) at the end of September 2010 in a comparison of a traditional pan trap with the new PAFIC trap that I developed (see Chapter 5). This trap employs both the colour of the traditional pan trap (Samways *et al.* 2010) and the transparent plexiglass sheets (Perspex ™ (Maizey (Pty) Ltd, Cape Town)) of the flight intercept (Samways *et al.* 2010) trap mounted vertically on top of the pan. Flying insects are attracted to the colour of the pan and either fly accidentally into it directly or fly above the pan to inspect it for its nectar or pollen potential but then strike the Perspex ™ and subsequently fall into the pan. A dilute soap solution in the trap was used to reduce surface tension. After determining that PAFIC traps were efficient (and possibly more so than traditional pan traps – see Chapter 5) in catching bees the four main SSs (which also helped in demonstrating the

PAFIC trap's efficacy) were conducted to measure spatial and temporal aspects of bee recovery: (i) SS 4: October 2010 PAFIC trap sampling Deep (defined below) into Natural and PPR sub-sites of Site One, and adjacent to one mass-flowering bush, *Agathosma capensis* (L.) Dummer (Rutaceae); (ii) SS 5: as SS 4 but in Ecotone sub-sites (defined below) of Site One; (iii) SS 6: December 2010 PAFIC trap sampling Deep into Natural and PPR sub-sites of all five sites, and in the centre of flower concentrations (which were sparse compared to SS 4 and 5); and (iv) SS 7: as SS 6 but in Ecotone sub-sites at all five sites.

SS 1: Individual bee sweep netting

Sweep netting aimed to sample bees (called IBS netting here) in different study areas and sites for identification and analysis of bee species abundance and richness. This IBS netting should be distinguished from transect sweep netting in which the sweep netter walks along transects while sweeping the net back and forth without targeting any one visible insect. The protocol used differed in that I would stop at blooming flowers of various species and wait for bees and then attempt to catch them with a sweep net when the opportunity presented itself (bees landing close enough and staying long enough for a viably hopeful catch). I always tried to catch bees that were foraging for pollen and/or nectar (with mouth parts making contact with plant reproductive parts), during the bee sampling periods.

Each IBS netting session was at a different area on a different bush/flower and was performed between 09h00 and 18h00 on virtually cloudless (<20% cloud coverage), wind free or calm (wind speed < 7 km/h) days. Sampling dates were for 11 separate days from 29 March 2010 to 19 May 2010. In total, there were about 250 IBS netting sessions during which the total time spent waiting for bees (excluding time for bottling them and getting ready to IBS net again) varied between two and 13 min.

Although there were a variety of blooming plants (*Metalasia densa, Oxalis* spp., *Filicia filiformia* subsp. *filiformia, Serruria fasciflora*, various *Erica* spp., *Aspalathus* sp., *Caesia contorta*, *Ursinia* sp., *Euryops* sp., and *Senecio* sp.), the majority of sessions (209 sessions) were on the most abundantly blooming generalist species, *Metalasia densa* (Lam.) P. O. Karis (Asteraceae). *M. densa* was chosen for the majority of IBS netting sessions because it attracts a large variety of generalist species, many of them bees. This was preferable to a specialist pollination syndrome plant which would provide results with limited applicability to other taxa. IBS net sampling was performed in Sites One, Two, and Five out of the five sites in both Natural and PPR sub-sites. As *M. densa* was growing much more vibrantly, densely, and abundantly on the PPR sub-sites, more time was spent, as a result, IBS net sampling on the PPR sub-sites to obtain all species present irrespective of the size of the plant host. The flower of *M. densa* is typically light beige and the flowers form umbels which have pollenaria that extend out of the flower when in bloom and this (and possibly any nectar in extremely small nectaries within the flower) is highly attractive to anthophiles (Figure 3.2). The focus was on catching non-*Apis* bees.

For the remaining blooming plant species from which bees were collected with IBS netting, no comparative data between Natural and PPR sides for any one species were available as the flowers were patchy, sparse, and overall, few in number at the five sites, and this gave insufficient bee specimen numbers for statistical analysis. However, a number of bee species were caught with all of the above IBS netting effort that were not caught in any of the PAFIC trap samples and these are included in the total species list (Appendix 3).

All bees caught in sweep nets were chilled on site in vials in a cooler with ice or cold packs, and labels detailing the time, place, flower being visited, and weather conditions were placed with them in the vials. The insects were later killed in a freezer.

SS 2: Individual bee sweep netting

The second sweep netting session was an IBS netting study performed in conjunction with the Springtime SS 5 and 6 (discussed below) involving PAFIC trapping. Flowering plant species in bloom at the time included *Oxalis* spp., *Filicia filiformia* subsp. *filiformia, Euryops* spp., *Ursinia* sp., *Senecio* sp., Aizoaceae sp., various *Erica* spp., *Aspalathus* spp., *Caesia contorta*, and *Agathosma capensis*. Due to the PAFIC trap focus at the study time, IBS netting followed a simple protocol of attempting to catch new species of bees as they were observed during unoccupied times while working with PAFIC trap equipment. As the objective was to discover other species resident in Zachariashoek, no comparative data were actively sought between different study regions (Natural or PPR) nor was IBS netting necessarily limited to cloudless or non-windy days, though conditions were always noted and stored with successful bee catches. Bees were chilled on site and later stored as in SS 1. This study produced insufficient bee numbers to allow for any statistical analysis but a few bee species were caught that were not caught with PAFIC trap sampling and these are included in the final species list (Appendix 3).

SS 3: Pilot study to evaluate the effectiveness of Pan and Flight Intercept Combination (PAFIC) trapping

This session evaluated the PAFIC trap as an effective method for catching bees (see Chapter 5) Nine traditional pan traps were placed deep in the Natural area of Site One in the centroid of blooming flower patches for one 24 hour period during which the weather was mostly cloud free (< 10 % coverage) and calm (wind speed < 10 km/h) and nine PAFIC traps were placed likewise (with the Perspex ™ directed randomly) deep in the PPR area in the next 24-hour period towards the end of September (Springtime) with the same weather conditions. When only a few bee individuals were caught by the traditional pan trap and many more (see Chapter 5) were caught with the PAFIC trap, it was concluded that PAFIC traps were effective at catching bees and possibly/probably more so than traditional pan traps. Previous experience with my sweep

net had shown that an experienced sweep netter will easily catch more than five bee individuals in an 8 hour day of IBS netting (typically catching 15 to 25 in a day depending on the site and available nectar and pollen forage).

As the objective of the remaining planned trap SS's was to compare bee species and abundance data between Natural and PPR sub-sites, it was only necessary to prove that the PAFIC trap was effective in catching bees but not necessarily significantly more so than traditional pan traps. With the timing and trap placement being consistent at both sub-sites, the data acquired would be statistically analysable. Ultimately, however, the higher number of bees caught with PAFIC traps, in the light of the remaining SS's (see below), indicated a good possibility that PAFIC traps are more effective than traditional pan traps (see complete discussion of this in Chapter 5).

SS 4: Springtime deep mass-flowering PAFIC trapping

The PAFIC trap sampling in October 2010 was performed at Site One of the five study sites for four 24-hour periods of usually cloudless (cloud cover < 20%) and wind-free or calm weather (wind-speed < 10 km/hr). This timing coincided with the main blooming season of the fynbos flora, with many plant species being in bloom (including, but not limited to, the species mentioned below for the December sampling). One species, *Agathosma capensis*, was blooming all together in a mass-flowering event in the study area. Choice of Natural sub-site or PPR sub-site locations for the PAFIC trap sampling was temporally alternated over these four 24-hour periods (first sampling in PPR, second in Natural, third in PPR, and fourth in Natural). It was initially intended that all four 24-hour sampling periods be consecutive but inclement weather made this impossible, so only the first two 24-hour periods were consecutive.

PAFIC traps were placed in immediate adjacency to the blooms of in-flower *A. capensis* bushes with the flat side of the Perspex ™ facing the center of the blooming flowers on the bush. Nine traps (three blue, three yellow, and three white) were placed at distances >60 m on both Natural and PPR sub-sites of Site One (Figure 4.1 (i)) adjacent to the most prolific flowering individuals of *A. capensis* (Figure 4.1 (ii)). The distances were mostly from 60 to 140 m, although five PAFIC traps were placed at about 200 m into the Natural sub-site due to lower availability of *A. capensis* plants on the Natural sub-site. A Latin square formation was attempted for the placement of traps but significant deviation from this pattern occurred due to the locations of the *A. capensis* plants in bloom.

The combination of pan trap and flight intercept trap necessitates a look at both methods for understanding what the PAFIC trap's minimum separating distance should be to avoid pseudo-replication. In this regard, Droege *et al.* (2010) show quite effectively that inter-trap effects plateau, for pan traps in a variety of ecologically differing areas, to zero at approximately three to five m and accordingly advise five m as sufficient for avoiding pseudo-replication.

For flight-intercept traps, unfortunately, no such research on safe distances to avoid pseudo-replication has been performed. Nevertheless some sense as to the importance of avoiding pseudo-replication has made flight-intercept trap users at least mention the distances which separated their traps – e.g. 5 m (Trisnawati & Nakamura 2008), 10 m (Carrel 2002), 11 to 290 m (Ulyshen & Hanula 2004; horizontally, though vertically paired traps were separated by 18.8 ± 0.95 m), 20 m (Hyvärinen *et al.* 2006, Pitkänena *et al.* 2008), 25 m (De Groot & Nott 2001), and "an average of 32 m" (Sverdrup-Thygeson & Birkemoe 2009). All of the traps for these studies (except Carrel (2002)) were for total flight-intercept (window) traps with collision areas varying between 1200 and 4800 $cm^2$.

It is reasonable to say larger flight-intercept traps need more inter-trap separating space to avoid pseudo-replication than smaller flight-intercept traps. This is reasonable from a simple "thought-experiment" of considering the extreme case of the two following pairs of flight-intercept traps: the first are two parallel, very small (area: 60 $cm^2$ = 6 cm X 10 cm) flight-intercept traps separated by 30 cm and the second are two parallel, very large (area: 60000 $cm^2$ = 200 cm X 300 cm) flight-intercept traps separated by the *same* 30 cm distance. A completely isolated single trap the size of the above very small one is not reasonably expected to have many (if *any*) more insects than either of the two parallel ones of this smaller size. A completely isolated single trap the size of the above very large one, however, will obviously have many more insects (perhaps twice as many) as either of the two parallel ones of this larger size. This example is an extreme case of course, but this only begs the question: what is a reasonable separating distance for the more common sizes of flight-intercept traps as used in most flight-intercept trap studies? In the absence of research similar to Droege *et al.* (2010) for flight-intercept traps I opted to have a minimum distance of 15 m – 50% larger than the 10 m used by Carrel (2002), who's trap had a surface area comparable to the PAFIC trap of my study. This distance, too, was used as a guideline for the minimum, and in practice did not occur more than thrice out of the 26 PAFIC traps in this SS. The remaining inter-trap separation distances were significantly higher (typically being about 20 to 40 m, though in one case it was 100 m). Further, 15 m neighbouring traps were always of different colours unless this was unavoidable due to *A. capensis* distributions (in which case such neighbouring traps of the same colour were always at least 30 m apart) in order to further decrease likelihood of inter-trap effects.

(Figure 4.1)

I chose all trap locations individually, hammered an iron pole into the ground (to the extent possible given the often rocky and steep topography) and placed the PAFIC traps in black garbage bags tied to the poles. These locations were pre-selected to the October trapping dates. As this study encompassed some non consecutive 24-hour time periods, *A. capensis* bushes were always re-evaluated on non-consecutive chosen sampling dates and if a designated *A. capensis* flowering bush had visibly gone past its peak flowering stage (with petals that were all drying out) then an alternative nearby *A. capensis* bush with fresh flowers was selected, and the trap was affixed immediately adjacent to it instead.

Normal PAFIC trap methodology would include the 90º rotation of the PAFIC trap in keeping with the flight-intercept element of the trap. In the case of pre-selected blooming *A. capensis* bushes, this would mean completely relocating the trap to the right or left of the bush in order for a 90º rotation of the trap to still allow the flat side of the Perspex ™ of the trap to face the center of the flowers of the bush. This was not possible for the October sampling, however, as the locations of suitable *A. capensis* bushes often had inadequate soils for firmly holding the traps, forcing me to use (often extensive) support from other iron poles, sticks, cable ties, and surrounding vegetation to keep the trap firmly in place and at the correct angle.

For all PAFIC trap studies, catches were taken to a laboratory where the aqueous detergent solution was disposed through a fine mesh plastic sieve and the retained insects stored in 70% ethanol.

SS 5: Springtime Ecotone mass-flowering PAFIC trapping

Methodology here was as for SS 4 except that there were only four yellow PAFIC traps placed at 10, 20, 30, and 40 m from the Natural/PPR boundary (the Ecotone). Due to the availability of suitable flowering *A. capensis* bushes the sequence of four traps was not necessarily linear from the boundary (Figure 4.1 (i)).

SS 6: Late Spring/Early Summer deep sparse-flowering PAFIC trapping

The PAFIC trap sampling of late December was done in all five sites for two 24-hour periods (19-20 and 20-21December 2010), the late blooming season of the CFR. Flowering plant species included a yellow *Ursinia* sp., a *Euryops* sp., *Senecio* spp., *Agathosma capensis*, *Aspalathus* spp., *Berkheya* spp., *Caesia contorta*, *Cliffortia ruscifolia*, *Diosma meyeriana*, *Erica* spp. (*E. abietina* subsp. *aurantiaca*, *E. articularis*, *E. bicolor*, *E. imbricata*, *E. nudiflora*, *E. plukinetti*, *E. rigidula*), *Filicia filifolia* subsp. *filifolia*, *Helichrisum* spp., a *Lobostemon* sp., *Metalasia densa*, *Oftia africana*, *Phaenocoma prolifera*, *Prismatocarpus brevilobus*, *Protea nitida*, *Protea scorzonerifolia*, *Protea repens*, a *Salvia* sp., *Serruria fasciflora*, and another *Serruria* sp. The plants that were blooming formed generally sparse flower patches and none were mass-flowering at the time.

PAFIC traps were placed at the largest and most concentrated patches of flowers (Figure 4.2). Traps were placed at the same height as the flowers for maximum efficiency (Tuell & Isaacs 2009). As In SS 4, nine PAFIC traps, three yellow, three white, and three blue, were placed deeper than 60 m but never more than 220 m from the Natural/PPR boundary (Figure 4.1 (i)). Distinct patches of blooming flower patches were not always available for all PAFIC traps, so a few PAFIC traps were simply placed in the general vicinity of blooming flowers wherever they were found. PAFIC traps were placed within 1 m of the centroid of flower patches.

(Figure 4.2)

Traps were never closer to each other than 15 m to avoid non-independence of trap catches (pseudo-replication) and such "adjacent" traps were never of the same colour unless this was unavoidable due to flower patch distribution, in which case such adjacent traps of the same colour were at least 30 m apart.

For the December PAFIC trap sampling there was no focus on optimizing the orientation of the PAFIC trap's Flight-Intercept element for any one plant species, mass-flowering or otherwise, to aid in catching anthophiles. A 90º rotation was easily peformed by rotating the trap on the iron bar used to support it during sample collection after the first 24-hour period and fixing the trap at this angle for the second 24-hour period. The PAFIC trap Perspex ™ sheets were directed down the slope on one day and perpendicular to the slope on the other day with direction of the trap Perspex ™ sheets alternating between adjacent traps on a given day.

Sampling sesson 7: Early Summer Ecotone sparse-flowering PAFIC trapping

As in SS 5, but following the vicinity-of-flower-patch placement strategy of SS 6, four yellow traps were placed at 10, 20, 30, and 40 m from the Natural/PPR boundary (Figure 4.1 (i)) for two consecutive 24-hour periods (19-20, 20-21December) near the most concentrated patches of flowers (Figure 4.2)

*Insect sorting and identification*

All bee samples from PAFIC trapping and sweep netting, were meticulously sorted to morphospecies, and a reference collection expertly identified. A voucher collection remains in the Entomological Museum, Department of Conservation Ecology and Entomology, Stellenbosch University.

**Statistical analysis**

For SSs 4 and 5, insufficient number of bee individuals was trapped to allow for full statistical analyses, so in this chapter statistical examination was only done for bee data from SS 6 and 7, with additional bee species from SS 4 and 5 included in the list of bee species (Appendix 3).

Species accumulation curves were generated using EstimateS Version 8.2.0 (Colwell 1997) to assess sampling sufficiency for SS 6 and 7. Differences between PPR and Natural sub-sites were analyzed across all distances from the Natural/PPR boundary using Wilcoxon's matched pair tests as data were not normally

distributed; species with < 4 occurrences were removed prior to statistical analysis. Wilcoxon's paired t-tests were used to compare species richness between Natural and PPR sub-sites. In addition, two regression analyses (one for Natural sub-sites and one for PPR sub-sites) were performed for yellow PAFIC trap total bee species abundance vs. distance from the Natural/PPR boundary.

All locally rare species for which there were < 4 occurrences were pooled and examined together (referred to as Single/Double/Tripletons). A Wilcoxon's paired t-test was used to see if there were any differences between the PPR and Natural sub-sites for these locally rare species as a group.

All statistical analyses were performed with STATISTICA version 11 (StatSoft, Inc. (2012).

**Results**

*Raw bee species diversity*

The total number of species sampled was 63 (see Figure 4.3 (i) and Appendix 3). Assuming that the seven male specimens of un-described new species were gender partners of other congeneric female undescribed new species, the number then becomes 56 species of bees sampled using all bees caught in the Zachariashoek valley with both PAFIC traps and sweep nets. In a separate pilot study of the Wemmershoek valley, about six km away, another five species of bees were caught and identified (personal observation, unpublished data). Southern African bee species commonly are distributed over very wide areas (Connal Eardley, personal communication) so it is reasonable to add these five species to conclude that the Zachariashoek valley has at least 61 species of bees. Given the temporally incomplete nature of the sampling (PAFIC trapping for two 24-hour periods on each of Natural and PPR sub-sites for site one in October; PAFIC trapping for two 24-hour periods one each of Natural and PPR sub-sites for all five sites in late December; IBS net sampling for 11 days in March, April, and May; and opportunistic IBS netting in October), and the topographically incomplete nature of the sampling (I did not do any sampling in riparians, mountaintops, Alluvial valley floors, or cliff faces) the number of species being 56 (or 61 including the Wemmershoek species) is conservative.

(Figure 4.3)

A total of 37 species (or up to 44 if unique male specimens assumed as gender partners of other species are in fact from other species) from this sampling have previously not been described and are identified only to the level of genus but distinguished from other species from the same genus with numbers (e.g. *Lasioglossum* sp. 1, *Lasioglossum* sp. 2, *Lasioglossum* sp. 3 etc.).

*Comparative bee species richness between Natural and PPR sub-sites*

Natural and PPR sub-sites had bee species richness values of 42 and 50 respectively. Of these, 29 species were shared between the two sub-sites, and 13 and 21 species were unique to Natural and PPR sub-sites respectively (Figure 4.3 (i)). In SS 4 and 5, during springtime mass-flowering PAFIC trapping, similar numbers of species were unique to the Natural and PPR sub-sites (Figure 4.3 (iii)). In SS 6 and 7, during early summer, sparse-flowering PAFIC trapping, almost equal numbers of species were caught uniquely in either PPR or Natural sub sites (Figure 4.3 (ii)). In SS 1, spring IBS netting (*Metalasia densa* - specific) (Figure 4.3 (iv)) six species were caught uniquely on the PPR sub-site, four species shared between the two sub-sites, and only one species was unique to the Natural sub-site. In SS 1, autumn IBS sampling indicates higher species richness on the PPR sub-sites. However, this must be qualified as more sampling effort was peformed in the Autumn session on the PPR sub-sites due to the greater abundance of the focal plant species (*M. densa*) for IBS netting in the PPR sub-sites.

A list of all new species (from all samplings, including both IBS netting and PAFIC trap sampling) is given in Table 4.1.

(Table 4.1)

*Sampling sufficiency*

The species accumulation curve did not reach an asymptote for the bee species sampled in this study (Figure 4.4). This was expected as sampling was not temporally or topographically thorough. Nevertheless the data of this study do provide insight for the bees that they represent and, in the absence of other sufficient data, they can be used to give tentative indications for what to expect for bees in general until further data are available.

(Figure 4.4)

*PAFIC trap colour*

In SS 6, there was marginally insignificant difference between abundance of bees in the nine traps of different colors (Yellow, White, Blue) in all zones of each sub-site (Kruskal-Wallis rank sum: $Chi^2 = 5.77$, $P = 0.056$). Difference between all (including Ecotone) Yellow PAFIC trap bee abundance data and the White PAFIC trap bee abundance data using a Wilcoxon's rank sum test (Mann-Whitney U-test for unmatched samples) was significant ($W = 1355.5$, $P = 0.021$) (Figure 4.5). There was no significant difference between Yellow and Blue PAFIC traps ($W = 905.5$, $P = 0.2755$) and a Wilcoxon's signed rank test for matched pairs between Blue and White PAFIC trap abundances also showed no significant difference ($t = 214$, $P = 0.165$).

(Figure 4.5)

*Natural vs. PPR sub-site bee abundance*

Grouping all bee abundance PAFIC trap data (Ecotone and Deep data), a Wilcoxon's signed rank test for matched pairs indicates that Natural sub-sites had significantly higher bee abundance than PPR sub-sites ($t = 543$, $P = 0.0038$) (Table 4.2). Natural sub-site Ecotone had higher bee abundance than PPR Ecotone ($t = 137$, $P = 0.0044$) (Table 4.3) but Natural sub-site Deep zones did not have significantly higher bee abundance than PPR Deep zones ($t = 632$, $P = 0.1100$, Table 4.4).

(Tables 4.2, 4.3, and 4.4)

The Wilcoxon's Rank Sum test was used on individual bee species abundance and on the total bee species abundance to test for differences in abundances between Natural and PPR sub-sites with all data grouped (Ecotone or Deep zones pooled), for data in Ecotones only, and for data in Deep zones only. For individual species, only one species had a significantly higher abundance (no matter how data were grouped: Ecotone and Deep zones' data pooled together or analysed separately) in Natural sub-sites over PPR sub-sites (*Ceratina braunsii*, $t = 195.5$, $P = 0.0006$, Ecotone and Deep data pooled together). When bee species were grouped according to genus or family, there was a significantly higher abundance of bees (whether Ecotone and Deep zones' data were pooled or kept separate) in the Natural sub-sites for *Ceratina* spp. and for the family Apidae (*Ceratina* spp.: $t = 176$, $P = 0.0004$; Apidae: $t = 191$, $P = 0.0003$, Ecotone and Deep data pooled together).

Data from the different trap colors were analyzed separately. There were no significant differences in individual or total species abundance between Natural and PPR sub-sites for Blue PAFIC traps (the closest was for Apidae: $t = 61$, $P = 0.2597$). White PAFIC traps did show significantly higher Natural sub-site abundance for total bee abundance ($t = 99.5$, $P = 0.0263$), Apidae abundance ($t = 52.5$, $P = 0.0114$), and abundance of *Ceratina braunsii* ($t = 63$, $P = 0.0078$).

Regression of bee abundance vs. distance from Natural/PPR boundary showed no relationship in the PPR sub-sites ($R^2 = 0.002$, $t = 0.361$, $P = 0.719$), although there was an increase in bee abundance with increasing distance into the Natural sub-sites ($R^2 = 0.106$, $t = 2.727$, $P = 0.008$) (Figure 4.6).

(Figure 4.6)

## Discussion

### *Effect of PAFIC trap colour*

The yellow trap always had the highest number of bees suggesting that it is the best colour to use under the conditions of this study. This is in agreement with research performed by others (Vrdoljak 2010, Kehinde 2011).

### *Sampling sufficiency*

The data on bees from SS 4 and 5 were not sufficient to analyze statitistically and as they only were collected from one sampling site using a different sampling method (placed immediately adjacent to mass-flowering *Agathosma capensis*) they were not included in the SAC generated by the bee data of SS 6 and 7. The SAC generated by EstimateS (Version 8.2.0) (Colwell 1997) did not fully approach an asymptote indicating insufficient sampling. It is proposed that if sampling had been done throughout the year and in a topographically complete way (sampling riverine areas, the valley basin, and the mountain tops) the SAC should show sampling sufficiency. Nevertheless, in the absence of more complete data, the conclusions drawn for the bees identified in this study are used to draw tentative conclusions for bees in general in Zachariashoek and the CFR.

### *General inferences*

Raw bee species richness (Fig. 4.3 (i)) indicates higher species richness in PPR sub-sites (50 species) than Natural sub-sites (42 species). This skew is from the IBS net sampling effort, which was mostly done in Autumn (SS 1) on *Metalasia densa* with comparatively little sampling on other flowers or in other seasons (SS 2). *M. densa* grew much more robustly, vibrantly, and plentifully on the PPR sub sites (personal observation, unpublished data), which led to a skew in IBS net sampling effort which in turn skewed raw bee species richness in favour of the PPR sub-sites (Fig. 4.3 (iv)).

The bias of this skewed sampling effort is possibly accentuated by the fact that large concentrations of mass-flowering species (of which *M. densa* is an example) attract a larger and more species rich insect anthophile assemblages (Westphal *et al.* 2003). For this reason, it is difficult to correct for the IBS net sampling of Autumn (SS 1) by, for example, dividing the total number of species by the time spent sampling as such corrective measures still do not account for the variations in attractiveness due to differing flower concentrations.

The IBS net sampling of October 2010 (SS 2) was performed on different flowers during other intensive PAFIC trap sampling work. These data, not included in Figure 4.3 (although species unique to SS 2 are in the list of bee species in Appendix 3), also have no reliable spatial comparison as different plant species may attract different bee species assemblages and bee flower-visitor abundance (personal observation, unpublished data).

Although IBS net data are problematic for spatial comparison, the PAFIC trap sampling in October and December 2010 (SS 4, 5, 6, and 7) do allow spatial comparison of raw bee species richness as a balanced spatial sampling strategy was used in these sessions. These experiments, temporally grouped in Figure 4.3 (ii) and 4.3 (iii) according to season (Spring: SS 4 and 5 – Fig. 4.3 (iii); Late spring/Early summer: SS 6 and 7 – Fig. 4.3 (ii)), show basically equivalent bee species richness in Natural and PPR sub-sites: (a) in spring (October), seven species were unique to, and 13 species were shared by, both Natural and PPR sub-sites in October; (b) in late spring/early summer (December), four and five species were unique to Natural and PPR sub-sites, respectively and 16 species were common to both sides.

The near-equivalence in spatial raw bee biodiversity is in contrast to spatial comparison of abundance of bees species individually, bee species complexes (for species not consistently differentiated and thus pooled to form a consistently recognizable taxonomic unit), and for bees grouped according to genus or family. No matter how bee species are or are not grouped, the result is nearly unanimous: except for one case (discussed in detail below) bee abundance was always higher in the Natural sub-sites than in the PPR sub-sites whenever there was a statistical difference in abundance (Tables 4.2, 4.3, and 4.4).

A possible explanation for this is that soils suitable for nesting in the PPR sub-sites are a limiting resource with much of it too disturbed to afford natural bee-assemblages and bee assemblage abundance normal nesting space. This is based on the fact that the majority of solitary (non-Apis) bees make their nests in soil (Houston 1969, Knerer & Schwarz 1976, Michener 1964).

The soil in Natural areas was noticeably more compact than the comparatively loose soil of PPR areas (Table 2.3). Such soil looseness could lead to a variety of nest-making problems for bees such as nest instability, easier access by biotic bee enemies (which are many, both epigaeic and endogaeic: Wcislo & Cane 1996), inappropriate soil biota, fungi, etc. Although soil is the most common environment for bee nests, some bees also make their nests in specific plant species such as *Aloe ferox* (Gess 1981, Gess & Gess 2014). If vegetation in PPR zones is depauperate (see Chapter 2) it is possible that such bees may not have the appropriate host plants for nest building.

Another possible explanation for lower bee abundance in the PPR areas is that, assuming some species have no difficulty making nests in PPR soil, depauperate flora in PPR sub-sites provide inadequate nutrition to support such potential bee inhabitants. Poorer plant assemblage richness for common species has been verified by my study of comparative common plant species between the PPR and Natural areas (see Chapter 2). Since it is known that a variety of plants is required to provide forage support for a variety of

pollinators (Scott-Dupree & Winston 1987, as mentioned by Wilcock & Neiland 2002) one would therefore expect inadequate nutrition in PPR areas. Such inadequate pollen and/or nectar providing plant assemblages may also result from disturbed soil being inadequate for them.

*Causes of soil disturbance*

All this begs the question what caused the soil to be so disturbed, and, if the plant species provide inadequate nutrition, why was there not a natural succession of plant species? The answer to this is complex but here it probably involves a combination of soil disturbance due to the synergy of three things: (i) the epigaeic extinction of fynbos plant life in the shade of the pine trees and concomitant extinction, to some extent, of endogaeic plant life in the seedbank and plant root structures, (ii) the high annual litterfall of pine stands (with mature pine stands nearly double that of mature montane fynbos (Versfeld (1981), as reported by Richardson & van Wilgen (1986)) which could have buried fynbos topsoil (and seedbank) and prevented it from receiving typical stimuli for germination, and (iii) the probably hotter-than-usual nature of the fire in the afforested area compared to the type of fire typical of the fynbos matrix. As several factors may be involved, by default, they have to be grouped together and referred to as post-pine recovery (PPR).

This last factor of soil disturbance via unusually hot (i.e. higher fire intensity and higher fire severity) fire is a factor that distinguishes the accidental 1999 Zachariashoek fire from other deliberately lighted pine forest fires (e.g. the "burn standing" fire treatment of Holmes *et al.* (2000)) in other regions of the CFR. Such deliberately lighted fires are usually in the cooler and wetter winter months for various management purposes and are less intense and severe than accidental hot and dry season fires like the one here. As fires in invaded areas typically burn at a higher temperature than non-invaded areas as a result of a higher fuel load (Brooks *et al.* 2004) it means that the fire here was probably hotter in the area of the forest stands. However, there is strong suggestion that it was much hotter in the pine forest than in the fynbos matrix. This was shown by pine stumps being burned right down to the level of the ground with even the endogaeic root bulb of the tree being charcoal to 10 cm beneath the level of the ground at Site Five. Also, there is evidence of extreme heat from a melted gearbox of a vehicle that was trapped in the fire, bearing in mind that the melting point of iron is 1538ºC and that of aluminium is 660ºC. Such extremely hot fires have proven to kill the seedbank, plant bulbs etc., at a significantly deeper level than regular fynbos fires (Cilliers *et al.* 2004), and possibly disturb the soil to a greater extent than that engendered by pine forest shading and litterfall alone. A confounding factor is that the pine trees' high litterfall could have insulated the original fynbos soil well if it did not burn completely (Neary *et al.* 2005), an observation that has been suggested for the litterfall of other trees invasive to fynbos such as *Acacia saligna* (Holmes & Cowling 1997). This possibility seems remote, however, given that the fire occurred in summer when the litterfall is in its driest and most flammable state.

Another complicating factor in the fire here is that it is possible for some regions of the valley to have experienced extreme heat (high fire intensity and high fire severity) while others experienced moderate or low heat (low fire intensity and low fire severity - possibly even lower than the fire in the fynbos matrix). This is because an individual pine forest fire can have varying fire intensities and severities in the area of the burn as a result of microclimate variations in moisture, wind, and air-flow in the burn area (Neary *et al.* 2005).

Because of all these factors, it is difficult to ascertain exactly how much hotter the Zachariashoek pine-afforested area fire actually was, the spatial distribution of such hotter fires throughout the valley, and/or the amount of heat transmitted to the original fynbos soil in the afforested portions of the valley. Nevertheless, the overall/general evaluation, given that fires in invaded areas are usually hotter due to higher fuel loads (Brooks *et al.* 2004) and that original surviving indigenous seedbank is much depleted by the heat in stands of alien pine trees (Cilliers *et al.* 2004), is that the fire here was probably significantly hotter than regular fynbos fires and deliberate winter-season pine fires across much of the five study sites.

*Inferences about bee species*

There is little research on which bee species in South Africa are more (or less) associated with disturbed areas. In the absence of research there is the opinion of experts, and in this regard there are a few genera that are generally associated with disturbed areas, some that are most typically from pristine areas, and many that have been commonly observed in both. *Lasioglossum* spp. have been reported commonly from disturbed areas and are therefore expected from the PPR sub-sites in my study. The single *Spinanthidium* sp., the two *Colletes* spp., and the two *Hylaeus* spp. are all of genera that have been reported mostly from natural/pristine areas (in my study therefore expected from the Natural sub-sites). All 42 other species are from genera found in both disturbed and pristine areas (in my study therefore expected from the Natural and PPR sub-sites) (Connal Eardley, personal communication). These previous findings need to be taken in the context of the different methodology used by researchers to gather specimens for expert identification – mostly from pan traps and sweep nets. The former method might easily under-represent certain species as the PAFIC trap catches more species and numbers of bees (see Chapter 5). Sweep netting is also significantly affected by the observer/catcher bias and by the observer effect (see Chapter 3), the latter of which which requires a significant waiting period that is rarely performed in bee netting experiments (but see Pontin *et al.* 2006 for a study with a one-minute waiting period).

Only SS 6 and 7 (December PAFIC sampling) had sufficient numbers of bees that allowed statistical comparisons between Natural and PPR sub-sites. In the case of species from these SSs in genera expected from the PPR sub-sites, only three (*Lasioglossum* spp. 3, 4, and 15) had sufficient data for Natural – PPR comparison. These three *Lasioglossum* spp. were collected in both Natural and PPR sub-sites meaning they were in "partial agreement" or "partial disagreement" with the expected result. "Partial disagreement" is

perhaps more correct here as these species had higher abundance on the Natural sub-sites (although, technically speaking, not statistically higher abundance). None of the species reported to come mostly from pristine areas (*Spinanthidium callescens* (Cockerell) sp., the two *Colletes* spp., or the two *Hylaeus* spp.) had sufficient data for statistical analysis in SS 6 and 7. Of the four species expected to come from both Natural and PPR sub-sites, only four in SS 6 and 7 were caught in sufficient numbers for statistical analysis (*Ceratina braunsii* (Eardley & Daly), *Braunsapis albipennis* (Friese), *Halictus* (*Seladonia*) sp. 1, and *Halictus* (*Seladonia*) sp. 2). All of these were caught in both Natural and PPR sub-sites – in agreement with predictions. This means that out of seven statistically analysable species (two (*Lasioglossum* spp. 3 and 4) of which were grouped in a complex), four were found as predicted by available information from experts while three, were in "partial agreement/disagreement" with expert prediction being caught in both PPR and Natural sub-sites.

*Bee species with counts too low for statistical analysis*

The remaining bee species (from SSs 1 to 5) were not caught in sufficient numbers to allow statistical comparisons between Natural and PPR sub-sites. However, even in the absence of statistically rigorous evidence, their absence/presence provides some valuable information. Of the 34 species (38 including males discounted as gender partners of other females if they are new species) that are predicted to come from both disturbed and pristine areas, 11 were caught on both Natural and PPR sub-sites and 19 were in "partial agreement/disagreement" (23 including discounted males) as they were caught on one of the two kinds of sub-sites; of the 14 (*Lasioglossum*) species that were predicted to come from mostly disturbed areas, only two (*Lasioglossum* spp. 10 and 11) were caught only in PPR sub-sites, five were only in Natural sub-sites, and seven were in "partial agreement/disagreement" being in both Natural and PPR sub-sites. This makes a total of 13 agreements, 5 disagreements, and 26 "partial agreements/disagreements" of presence/absence bee data with the predictions of experts – a result on the whole similar to the four agreements and three "partial agreements/disagreements" of data of species that were sufficient for statistical analysis.

Although these conclusions are speculative given the low bee counts, the prediction of *Lasioglossum* spp. as associated with disturbed areas (mostly contradicted by this presence/absence evidence) needs to be understood in the context that bee data and specimens often come from agro-ecosystems (e.g. Eardley *et al.* 2006). This is due to the importance of bees for crops and the need for bee research in such areas. This "research area bias" can lead to a sampling bias causing higher incidence of certain species from such agriculturally disturbed areas leading to a perception of such species as disturbance-associated when actually they are present in natural areas but under-sampled there due to less sampling of bees in pristine areas. Additionally, most sampling is done with traditional pan traps and sweep nets, both of which can miss species that the PAFIC trap catches.

*Effects of bee motility*

As the Natural and PPR sub-sites were adjacent and compared to only about 100 m (and for one site up to 200 m) from the Natural/PPR boundary the question of bees residing in one side and flying to the other to forage arises. Bees are known to forage at distances that vary greatly depending on their size. Typically however, they have a maximum foraging distance of 150-600 m (Gathmann & Tscharntke 2002, Greenleaf *et al.* 2007), with the formula from Greenleaf *et al.* (2007) putting the maximum foraging distance between 170 to 860 m for bees with intertegular sizes of 1 to 2 mm. These maximum foraging distances are possibly underestimates for some species but, being maximum distances, there is reliable evidence that such distances are only flown by a few individuals within the population. The majority of the individuals for the focal species require nesting site to foraging site distances to be half of the maximum distances (Zurbuchen *et al.* 2010). With this in mind, distances between nests and foraging plants that are 80-300 m (or 90-480 m using Greenleaf *et al.*'s (2007) estimate for bees with intertegular sizes of 1-2 mm) are a better starting point to estimate whether bees nesting in one area could probably be seen foraging in another. This means that it is possible for bees that were caught on the Natural sub-sites to have nests in PPR sub-sites and vice versa. To rule out such incidences, it would be necessary to sample deeper within the PPR and Natural sub-sites. More importantly, such sampling should be performed on a long-term basis to verify that the differing levels of apifaunal abundance are not a stochastic variation from an ecological "snap-shot".

*Bee biodiversity in Zachariashoek*

With so little known about insect species (at most only 7-10% have been described (Samways 1993)), discovering new species of insects when doing entomological research in natural areas is not so much a factor of *if* as it is of *when* and *how many*. Of interest here is the total number of undescribed bee species sampled: 37 (potentially 43 if male specimens that appeared to be new species but were assumed as conspecific gender pairs of female specimens are, in fact, new species). As the Halictidae requires taxonomic revision (Connal Eardley, personal communication), this total number of new species may need further investigation. Also, since the definition of species is itself under much debate, and since insects have a comparatively fast microevolution (particularly in a relatively new environment such as the post pine-afforested areas for insects of the fynbos), it is difficult to confirm that all 37 new species sampled here are distinct and separate species. Nevertheless, this high number of new bee species points to a substantial taxonomic knowledge gap in the anthophiles in the Western Cape.

South Africa's authority on bees, Connal Eardley, after identifying the bees from the voucher collection of this study notes that typical bee sampling studies of disturbed and pristine areas normally have more Apidae, Megachilidae, and Colletidae species. He also notes that the collection in my study is

particularly rich in Halictidae, but that the assemblage collected has few specialist monolectic genera. This latter finding is consistent with expectations because the PAFIC sampling of October (SS 4 and 5) was next to a generalist pollination syndrome plant (*Agathosma capensis*), while the IBS net sampling was performed mostly on a generalist plant (*Metalasia densa*). Furthermore, in the case of the December sampling (SS 6 and 7), specialist bees may even be under-sampled, as they are not likely to visit coloured PAFIC traps for nectar/pollen owing to the traps' low resemblance to flowers and in view of their specialization in flower form. Nevertheless, based on the absence of bee species expected to be present in the area of this study, the estimate for the number of bee species in Zachariashok is approximately 100 (Connal Eardley, personal communication). This high number of bee species needs to be considered in its scale as Zachariashoek has a total area of about 3.5 $km^2$. For comparison, the Mediterranean olive groves mentioned in research by Potts *et al.* 2006, which had the highest bee species diversity in that study, had a total of 50 bee species.

Spatial scale is important in delineating and describing biodiversity. The area of Zachariashoek (3.5 $km^2$) translates the 100 species proposed for the valley to 28.5 species per $km^2$ (which translates to 0.29 species per ha assuming no overlap of species' territory). This figure changes if we estimate species per unit area by taking the actual 56 species discovered in Zachariashoek (for both the IBS net sampling studies and the PAFIC trap sampling studies) and dividing over the area in which the IBS net sampling and PAFIC trap sampling was done. The total sampling area was approximately 6.7 ha which translates to a bee species concentration of 8.3 species per ha. Translating this to number of species per $km^2$ scale we can go as high as 830 species per $km^2$, assuming total species turnover. The range of figures: 830 species per $km^2$ and 0.286 species per ha emphasizes the importance of correct use of spatial scale as neither of these numbers reflects an "on the ground" bee biodiversity reality.

*Insect diversity and the Intermediate Disturbance Hypothesis*

Grime's (1973) Intermediate Disturbance Hypothesis (IDH; see Fig. 4.7; also for explanation regarding attributing the hypothesis to Horn (1975), Connel (1978), or Huston (1979) see Wilkinson (1999)) is of questionable value as far as fire disturbance to fynbos plant species is concerned (Schwilk *et al.* 1997). However, for anthophile diversity variation with changes in fire disturbance regimes, it may still be of value as vegetation diversity is not necessarily a good surrogate for insect anthophile diversity (Vrdoljak 2010) and specifically not a good surrogate for bee diversity in Zachariashoek (see Chapters 2 and 6).

(Figure 4.7)

There are few data on how bee biodiversity responds to disturbance but the general trend from what can be gathered from experts, partially confirming IDH for bees, is that disturbed areas commonly have higher apifaunal species richness (Connal Eardley, personal communication) than pristine areas.

However, the complete application of IDH for bee biodiversity in Zachariashoek requires classifying the previously afforested area of the valley as "moderately" or "highly" disturbed. Such classification would only be possible with comparative data from areas confirmed as "highly" disturbed such as within the central confines of the nearby town of Franschhoek.

To rule out bee mobility confounding such bee species spatial comparison, such bee collection needs to be performed deeper into the PPR and Natural areas. This would only be possible in Zachariashoek at Site 1 because this was the only site that would allow for going 1 km + into the PPR area and 1 km + into the natural area while still maintining all other variables unchanged such as elevation, slope, aspect etc. Such a deep Natural – deep PPR comparison could also be done between the Zachariashoek alluvial valley and a nearby Haweqas/Limietberg Nature Reserve valley basin.

*Bee diversity in fynbos and in the CFR*

Given the difficulty in applying IDH to Zachariashoek, it is a complex issue whether other areas in the CFR would have higher or lower bee diversity than Zachariashoek. Assuming the IDH applies to bee diversity with fire disturbance, it is still difficult to know whether Zachariashoek lies on the scale of disturbance as "highly" or "moderately" disturbed. If Zachariashoek is "moderately" disturbed then other natural/pristine areas of the CFR near to Zachariashoek would hold lower numbers of bee species, but if "highly" disturbed then other pristine areas of the CFR nearby could conceivably hold higher numbers of bee species. If the conservative assumption is made for other nearby CFR areas in the Limietberg Nature Reserve then a pristine valley the size of Zachariashoek should hold at a most conservative estimate half the number of species estimated for Zachariashoek – 50 species of bees. As the Limietberg NR is not the most biodiverse region in the CFR, however, the very real possibility is that other more biodiverse regions of the CFR (e.g. the Kogelberg Biosphere Reserve) could have higher bee diversity than Zachariashoek.

Given that these numbers are all "worst-case" scenarios, these probably low estimates put the CFR, in my opinion, as a good contender for having the highest bee diversity in the world.

*Implications of lower bee abundances for pollination in PPR areas and inferences about specific bee species, genera, complexes, and families*

In the absence of long-term studies of bee diversity and abundance deep into Natural and PPR areas, the results of this study paint a picture that is worrying. Firstly, though decades of pine forest shading, pine forest litterfall, and hotter-than-usual (perhaps much hotter) pine forest fire do not cause a significant change in apifaunal species richness, this needs to be taken in the context of the fact that these disturbances do cause

a significant change in apifaunal composition. Secondly, and more disturbingly, they do cause a significant loss of natural apifaunal abundance.

My results show that there is a consistent statistically significantly higher abundance of total bees, Apidae, *Lasioglossum* sp. 15, *Ceratina braunsii*, (no matter how these are grouped or separated) in Natural sub-sites - whether one looks at Ecotones separately, Deep zones separately, or whole sub-sites together. This is also reflected in the differences in the raw number of bees: bee species, genera, families, or complexes were always higher in the Natural sub-sites (with one exception, for *Lasioglossum* complex 1 including *Lasioglossum* spp. 1, 13, &14). Despite these differences not always being statistically significant, in light of the fact that all, except one complex, were consistently higher on the Natural sub-site, this is highly indicative that areas disturbed by pine afforestation probably experience pollinator limitation to some extent as far as bees are concerned. Due to the functional centrality of bees in pollination, restoration of pollination as a keystone process needs to be considered for management of these areas.

Apidae, indicated as having statistically significantly higher abundance in Natural sub-sites, had insufficient data on one very important pollinator: the honeybee (*Apis mellifera* (L.) - in Zachariashoek probably *A. mellifera* subsp. *capensis*, though genetic verification is required for positive identification). Honeybees perform an inordinately large role in pollination despite only being 0.005% of the estimated 20 000 bee species in the world. Insufficient numbers of honeybees from PAFIC trapping were caught to allow any conclusion on differences in this important species' abundance between Natural and PPR sub-sites. Anecdotally, honeybees were observed to be plentiful in the PPR sub-sites during the IBS netting study of April and May 2010 on *Metalasia densa*. Further, as this plant does grow more abundantly on PPR sub-sites, this would indicate that honeybee mediated pollination limitation was not problematic for this abundant plant species.

Most pollination "interaction webs" are based on relatively rich connectedness and substantial temporal and spatial shifts (Kearns *et al.* 1998). However, if the general picture that there are simply fewer bees overall holds true as indicated by this study, then howsoever rich such interaction webs have the potential to be in post pine-afforestation-followed-by-pine-fire areas, there will simply be fewer bees to fill such pollination "webspaces".

Can other insect flower visitors from insect groups like wasps, flies (Diptera), and beetles (Coleoptera), fill the niche of bees? In the context of the evolutionary nature of pollination interactions being opportunistic (Kearns *et al.* 1998) on both the part of the pollinator and the pollinatee (the plant) one might hope so. However, as pollinator-plant interactions have such an ancient history (120 to 140 mya (Grimaldi 1999)) and involve extremely intricately evolved flower-seeking behaviours on the part of bees (Collett *et al.* 2013, DeIbarra *et al.* 2014, Giurfa 2007, Leonard & Masek 2014) along with the complication of co-evolving plant strategies in attracting pollinators (Moldenke 1979, Steiner & Whitehead 1991) such hopes are in all probability almost totally over-optimistic. The best is to quote Carl Sagan, "Really, it's okay

to reserve judgement until the evidence is in" and then stick to the precautionary principle and assume that other insect groups might likewise be limited in recolonizing post pine-afforested areas unless proven otherwise.

This study avoided pseudo-replication, and the conditions under which the sampling was done followed the established protocol for weather conditions (wind-free, relatively cloudless (<15% cloud cover), extremely hot (>35˚C) temperatures avoided) (Samways *et al.* 2010). Due to the importance of pollination for healthy plant recovery and the very significant findings of this temporally and topographically limited study, further study is required to expand the scope of knowledge. Questions need to be answered about bee population recovery in PPR afforested areas year-round and in other various geographically heterogeneous regions, especially riparian zones, seeps, mountain (and hill) tops, and valley basins. In the absence of available knowledge, the precautionary principle points, however, to assume that there is pollinator limitation with respect to bees in areas recovering from pine afforestation until proven otherwise.

The data from this study, being a "snapshot" for 13-year old fynbos, leaves an interesting window to see how the dynamics in the recovery of fynbos after 26 years and at 52 years might look. Long-term results like these would give a truer picture about recovery of bee populations given insect notoriety for large temporal and spatial variations in populations (Cane & Payne 1993, Williams *et al.* 2001).

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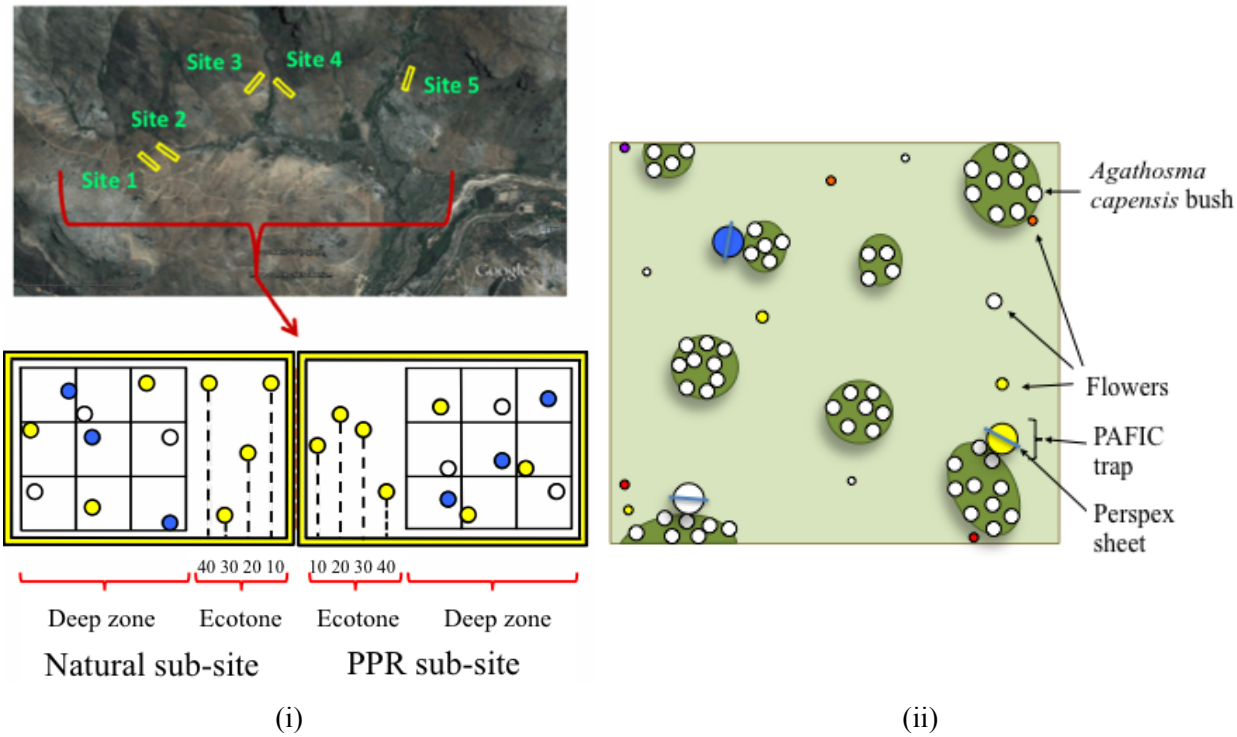



(i) (ii)

Figure 4. 1 (i) Top: Google ™ image of the research area with the five study sites. Bottom: diagram of an example of how the PAFIC traps were placed (deep zones not drawn to scale) in any one of the five sites – whether from the two October or the two December sampling Sampling Sessions (SS's). PAFIC traps in the Ecotone were at 10, 20, 30 and 40 m distances from the Natural – PPR boundary. Traps in the deep (60m + ) zone of each Natural or PPR sub-site were placed in a Latin Square to the extent possible but significant deviation from this was common due to locations of the bushes or flower patches. (ii) Schematic diagram of placement of PAFIC traps for SS 4 and 5 in October 2010 (Site 1 only): PAFIC traps in immediate adjacency to *Agathosma capensis* (L.) Dummer bushes. Note that PAFIC traps were much farther from each other than indicated in diagram (diagram not to scale).

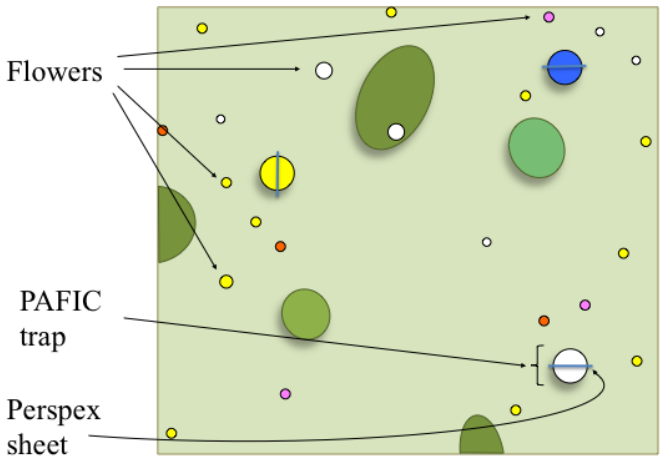



Figure 4. 2 Schematic diagram of placement of PAFIC traps for Sampling Sessions 6 and 7 in December 2010: PAFIC traps were in the approximate centre of most the concentrated flower patches which tended to be sparse. PAFIC traps were much further from each other than indicated in diagram (diagram not to scale).

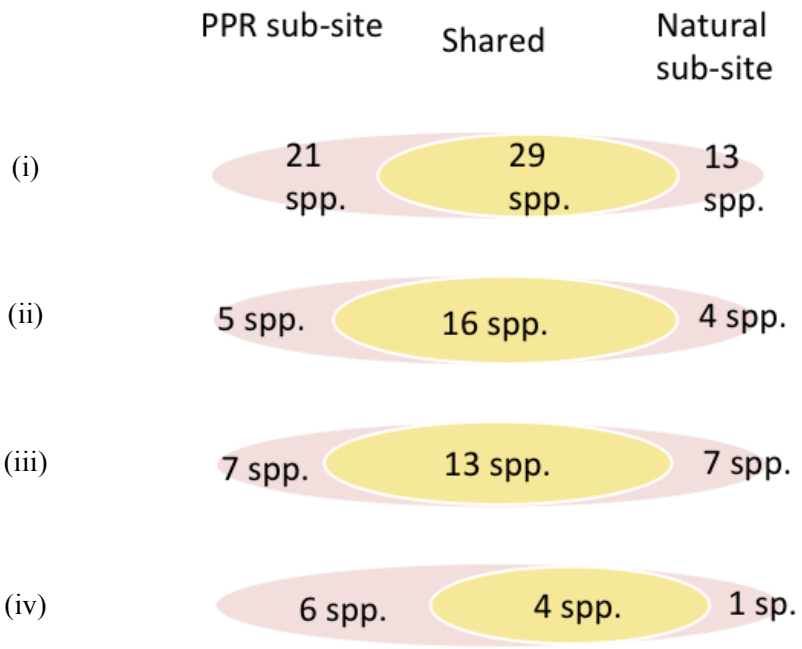



Figure 4. 3 Venn diagrams of bee raw biodiversity: (i) for all sampling – both Individual Bee Sweep (IBS) net samplings, and both Pan and Flight Intercept Combination (PAFIC) trap samplings, (ii) for the December PAFIC trap sampling only, (iii) for the October *Agathosma capensis* – specific PAFIC trap sampling only, and (iv) for the March/April/May *Metalasia densa* – specific IBS net sampling only.

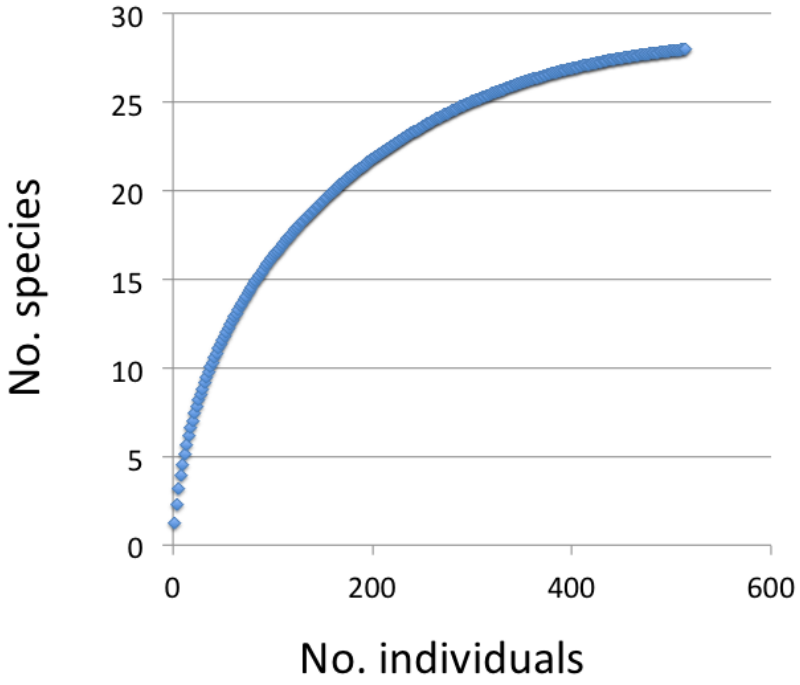



Figure 4. 4 The species accumulation curve performed by EstimateS (Colwell 1997) for the December Sampling Sessions (SS 6 and 7).

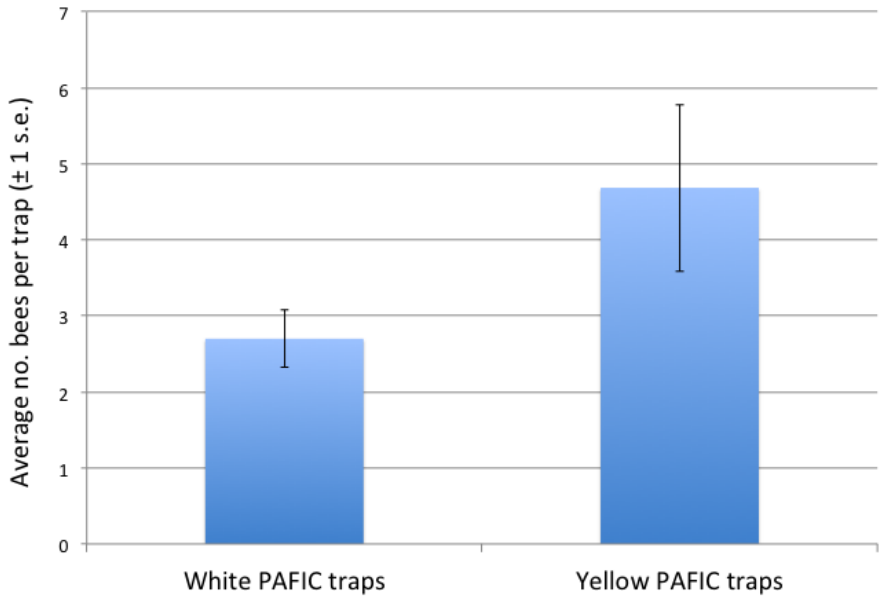
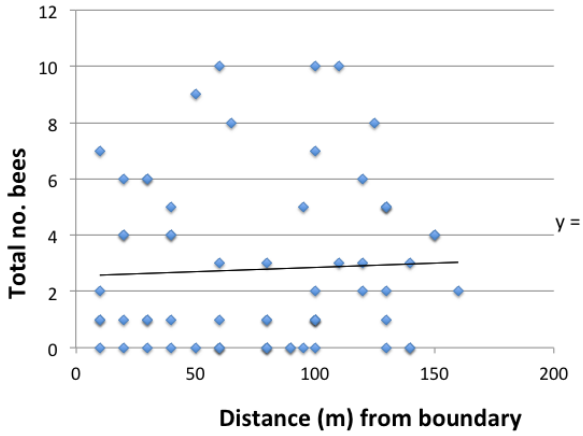
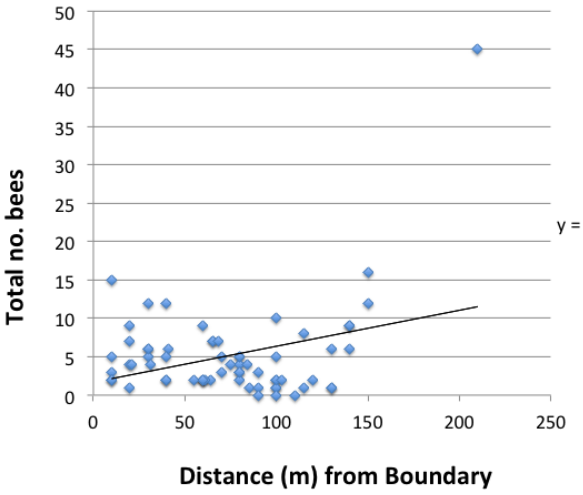



Figure 4. 5 Yellow Pan and Flight Intercept Combination (PAFIC) traps caught significantly more bees than white PAFIC traps.




(i)




(ii)

Figure 4. 6 Regression analysis of all PAFIC trap sampling in December 2010 showed no significant change of bee abundance with distance in the PPR sub-sites (i) (t =0.361, P=0.719) though there was a significant increase in the Natural sub-sites with distance (ii) (t =2.727, P=0.008).

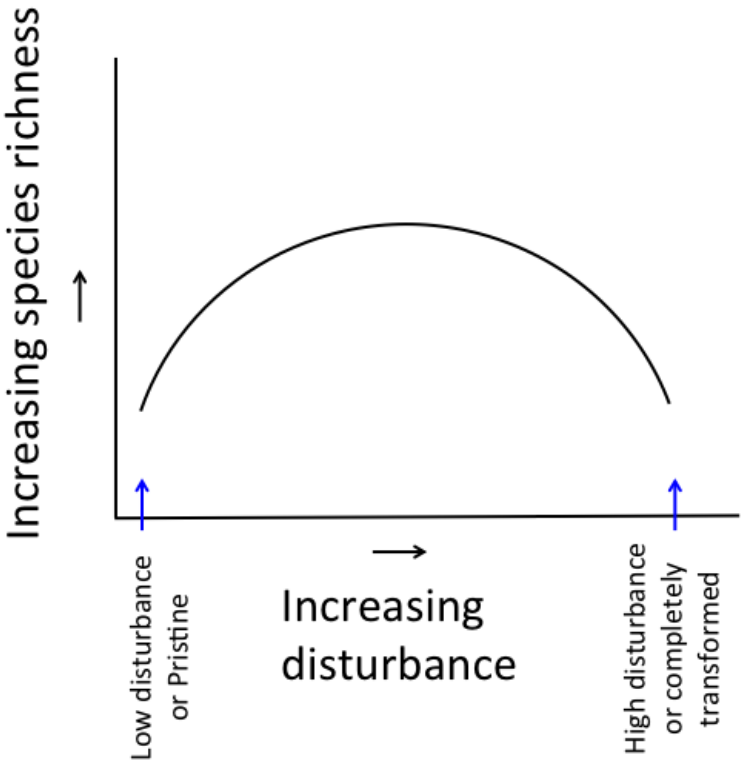



Figure 4. 7 The general version of the hump-backed Intermediate Disturbance Hypothesis (adapted from Wilkinson (1999)).

Table 4. 1 Numbers of new bee species discovered in Zachariashoek

| No. of species* | Genus | Family |
|---|---|---|
| 3 [5] | *Heriades* | Megachilidae |
| 2 | *Haetosmia* | Megachilidae |
| 2 | *Colletes* | Colletidae |
| 2 | *Hylaeus* | Colletidae |
| 1 | *Scrapter* | Colletidae |
| 17 | *Lasioglossum* | Halictidae |
| 1 [2] | *Nomioides* | Halictidae |
| 1 | *Ceylalictus* | Halictidae |
| 6 [10] | *Patellapis* | Halictidae |
| 2 | Unknown | NA |
| Total: 37 [44] | | |

* Total number of different female species; number in square brackets is the number of species if we assume unique looking male specimens were not conspecific to the other female specimens.

Table 4. 2 Pan and Flight Intercept Combination (PAFIC) trap bee abundance for both Deep and Ecotone zones and Wilcoxon's signed rank Sum test P-values. The total number of traps in each of the Natural and PPR sub-sites was 65.

| | Natural | | PPR | | P-value (t-value) |
|---|---|---|---|---|---|
| **Species/Species complex** | Total no. bees | Non-empty traps | Total no. bees | Non-empty traps | |
| *Halictus* (*Seladonia*) sp. 1 | 109 | 51 | 73 | 41 | 0.1752 (437) |
| *Halictus* (*Seladonia*) sp. 2 | 31 | 21 | 19 | 13 | 0.1204 (65) |
| *Lasioglossum* sp. 15 | 29 | 5 | 0 | 0 | 0.0579 (0) |
| *Lasioglossum* sp. 12 | 0 | 0 | 1 | 1 | NA |
| *Apis mellifera* subsp. *capensis* | 1 | 1 | 2 | 2 | NA |
| *Braunsapis albipennis* | 9 | 6 | 2 | 1 | 0.0975 (0) |
| *Ceratina aliceae* | 1 | 1 | 0 | 0 | NA |
| *Ceratina braunsii* | 77 | 50 | 26 | 19 | 0.0006** (195.5) |
| *Lasioglossum* complex 1 (sp. 1, 13 & 14) | 23 | 19 | 27 | 21 | 0.4771 (188) |
| *Lasioglossum* complex 2 (sp. 3 & 4) | 28 | 13 | 17 | 13 | 0.5325 (107.5) |
| *Xylocopa* sp. | 0 | 0 | 1 | 1 | NA |
| Single/Double/Tripletons | 25 | 18 | 13 | 10 | 0.0940 (61.5) |
| Total (for all spp. i.e. excl. fam) | 333 | 100 | 181 | 65 | 0.0038** (543) |
| **Genera/Family** | | | | | |
| *Halictus* (*Seladonia*) spp. | 140 | 61 | 92 | 47 | 0.1376 (424.5) |
| *Lasioglossum* spp. | 80 | 36 | 45 | 33 | 0.2602 (420) |
| *Ceratina* spp. | 78 | 51 | 26 | 19 | 0.0004** (176) |
| Halictidae | 220 | 79 | 137 | 60 | 0.0845 (521) |
| Apidae | 88 | 57 | 31 | 23 | 0.0003** (191) |

Table 4. 3 Pan and Flight Intercept Combination (PAFIC) trap bee abundance for Ecotone and Wilcoxon's Rank Sum test P-values.

| | Natural | | PPR | | P-value (t-Value) |
|---|---|---|---|---|---|
| **Species/Species complex** | Total no. bees | Non-empty traps | Total no. bees | Non-empty traps | |
| *Halictus* (*Seladonia*) sp. 1 | 29 | 14 | 17 | 13 | 0.1461 (96) |
| *Halictus* (*Seladonia*) sp. 2 | 15 | 11 | 11 | 7 | 0.3046 (25.5) |
| *Lasioglossum* sp. 15 | 11 | 3 | 0 | 0 | 0.1814 (6) |
| *Lasioglossum* sp. 12 | 0 | 0 | 0 | 0 | NA |
| *Apis mellifera* subsp. *capensis* | 0 | 0 | 0 | 0 | NA |
| *Braunsapis albipennis* | 0 | 0 | 0 | 0 | NA |
| *Ceratina aliceae* | 1 | 1 | 0 | 0 | NA |
| *Ceratina braunsii* | 30 | 21 | 9 | 8 | 0.0154** (114) |
| *Lasioglossum* complex 1 (sp. 1, 13 & 14) | 11 | 8 | 9 | 7 | 0.8048 (42.5) |
| *Lasioglossum* complex 2 (sp. 3 & 4) | 3 | 2 | 3 | 3 | 0.8501 |
| *Xylocopa* spp. | 0 | 0 | 0 | 0 | (4) |
| Single/Double/Tripletons | 12 | 9 | 4 | 3 | 0.0656 (38) |
| Total (for all spp. i.e. excl. fam) | 112 | 33 | 53 | 23 | 0.0044** (137) |
| **Genera/Family** | | | | | |
| *Halictus* (*Seladonia*) spp. | 25 | 20 | 12 | 17 | 0.1039 (69) |
| *Lasioglossum* spp. | 44 | 12 | 28 | 10 | 0.1428 (96) |
| *Ceratina* spp. | 31 | 22 | 9 | 8 | 0.0095** (105) |
| Halictidae | 69 | 24 | 40 | 21 | 0.0670 (82) |
| Apidae | 31 | 22 | 9 | 8 | 0.0095** (105) |

Table 4. 4 Pan and Flight Intercept Combination (PAFIC) trap abundance data for Deep zones, Wilcoxon's Rank Sum P-values

| | Natural | | PPR | | P-value (t-Value) |
|---|---|---|---|---|---|
| **Species/Species complex** | Total no. bees | Non-empty traps | Total no. bees | Non-empty traps | |
| *Halictus* (*Seladonia*) sp. 1 | 80 | 37 | 56 | 28 | 0.5015 (282.5) |
| *Halictus* (*Seladonia*) sp. 2 | 16 | 10 | 8 | 6 | 0.2663 (53) |
| *Lasioglossum* sp. 15 | 18 | 2 | 0 | 0 | NA |
| *Lasioglossum* sp. 12 | 0 | 0 | 1 | 1 | NA |
| *Apis mellifera* subsp. *capensis* | 1 | 1 | 2 | 2 | NA |
| *Braunsapis albipennis* | 9 | 6 | 2 | 1 | 0.0975 (10) |
| *Ceratina aliceae* | 0 | 0 | 0 | 0 | NA |
| *Ceratina braunsii* | 47 | 29 | 17 | 11 | 0.0155** (288) |
| *Lasioglossum* complex 1 (sp. 1, 13 & 14) | 12 | 11 | 18 | 14 | 0.1966 (27.5) |
| *Lasioglossum* complex 2 (sp. 3 & 4) | 25 | 11 | 14 | 10 | 0.4039 (104.5) |
| *Xylocopa* spp. | 0 | 0 | 1 | 1 | NA |
| Single/Double/Tripletons | 13 | 9 | 9 | 7 | 0.6094 (39) |
| Total (for all spp. i.e. excl. fam) | 221 | 67 | 128 | 42 | 0.1100 (632) |
| **Genera/Family** | | | | | |
| *Halictus* (*Seladonia*) spp. | 55 | 41 | 33 | 30 | 0.4795 (339) |
| *Lasioglossum* spp. | 96 | 24 | 64 | 23 | 0.7145 (234.5) |
| *Ceratina* spp. | 47 | 29 | 17 | 11 | 0.0155** (288) |
| Halictidae | 151 | 55 | 97 | 39 | 0.3928 (451.5) |
| Apidae | 57 | 35 | 22 | 15 | 0.0099** (334.5) |

# Chapter 5 – Development of a pan and flight intercept combination (PAFIC) trap for insect flower visitors


## Abstract

A new type of trap was developed for sampling flower-visiting (anthophile) insects for research. A sheet of Perspex was mounted vertically on a coloured pan trap to produce a Pan and Flight-Intercept Combination (PAFIC) trap that is much easier to use than the larger and more cumbersome traditional flight-intercept trap. A pilot test indicated that the PAFIC trap is effective in catching bees and more effective than traditional pan traps. Bee species richness was almost equivalent in spatial distributions between one test with the trap adjacent to a mass flowering species and another with the trap in sparser flower patches – indicating the PAFIC trap performs independently of degree of flowering in surrounding vegetation. I conclude that the PAFIC trap holds promise and deserves further rigorous testing to establish how it truly compares to traditional pan traps. Some discussion is offered on the mode of operation of the PAFIC trap in light of its placement close to a mass-flowering bush compared to placement in general vicinity of sparser flower patches.


## Introduction

Insect-mediated pollination (entomophily) is central for the production of 84% of food crops in Europe (Williams 1996). Pollination is estimated to contribute between $20 billion US (Kevan 1991) and $40 billion US (Pimentel *et al.* 1997) to US agriculture. Non-honeybee crop pollination may be as high as $6.7 billion US (Nabhan & Buchmann 1997). A significant number of non-honey bee pollinators are other bee species (Cane & Payne 1993).

The honeybee *Apis mellifera* L. has many subspecies unique to the various floristic regions and climates of the world. These subspecies perform a disproportionate amount of pollination in comparison to the actual number of bee species that exist in the world (estimated to be at least 20 000 species but there are possibly/probably many more) (Buchmann & Nabhan 1996, Wcislo & Cane 1996). Most species of bees are solitary, with a few eusocial and fewer truly social species with honeybee-hive like behaviour (Wcislo & Cane 1996). Solitary and non-*Apis* bees can enhance crop yields even where the honeybee is the main pollinator (Ramirez & Davenport 2013). Indeed, some crops are mostly pollinated by non-*Apis* bees, such as alfalfa (*Medicago sativa,* pollinated by *Megachile rotundata* Fabricius) and tomato (*Solanum lycopersicum*, pollinated by *Bombus* spp.) (Dafni *et al.* 2010, Velthuis & Cobb 1991, Winfree *et al.* 2008). The obvious economic importance of bees has led to a need for consistent and reliable monitoring methods for these insects.

Such methods are also important from an ecological perspective, as bees are also hugely important in the maintenance, balance, and conservation of natural ecosystems and play a central role in the pollination of approximately 80% of all flowering plants (Buchmann & Nabhan 1996). Reports of a looming global pollination crisis have been published for nearly two decades with decreases in honeybee populations and decreases in crop yields verified over this time (Aizen *et al.* 2009, Buchmann & Nabhan 1996, Garibaldi *et al.* 2011, Kearns *et al.* 1998, Holden 2006, Westerkamp & Gottsberger 2002). Insect populations are notorious for stochastic fluctuations (Cane & Payne 1993, Samways *et al.* 2010, Williams *et al.* 2001). This, predictably, leads to much difficulty in proving decreases in insect populations, necessitating the development of reliable and easy-to-replicate methodologies which can be applied for consistent long-term monitoring studies, of which there are currently few (but see Roubik 2001).

Foremost in the methods of monitoring bee populations are pan traps, sweep nets, and flight-intercept traps (Samways *et al.* 2010). Sweep netting for anthophiles is effective but limited by the Observer Effect (see Chapter 3) and observer bias. This is often countered with the use of pan traps, which have no Observer Effect or human behaviour bias and have been proven as one of the better methodologies for insect anthophile sampling (Campbell & Hanula 2007, Nielsen *et al.* 2011, Tuell & Isaacs 2009, Vrdoljak 2010, Westphal *et al.* 2008). Some research has, however, highlighted certain problems such as the presence of rich floral displays surrounding pan traps rendering them poor in attracting pollinating insects (Cane *et al.* 2000). Munyuli (2013) even found that sweep/hand nets are more effective, and that pan traps should be used as secondary/supplementary methods. In my own experience with my pilot study with nine coloured pan traps I only caught five bee individuals in a windless, cloud-free, 24-hour period, in the spring in Cape Floristic Region, South Africa. This was in the height of the blooming season with an accompanying presence of many bees – conditions under which an experienced sweep netter will easily catch more than five bee individuals in a single day (personal observation).

Flight intercept traps catch all flying insects, not only those that are visiting flowers. This introduces a potentially huge "by-catch" that is unnecessary, and in the context of insect conservation, arguably unethical (Samways *et al.* 2010). Such traps, which can often be large (e.g. 2 m X 1 m), are also burdensome to transport, and are labour intensive to set-up, maintain, and monitor.

In this chapter, I describe the development of a pan and flight-intercept combination (PAFIC) trap for insect flower visitors designed to overcome problems and biases associated with the traditional pan trap methodology. I then present the result of a single, unreplicated test of PAFIC and traditional pan traps in September 2010 to show that the PAFIC trap is effective and possibly more so than traditional pan traps. I used data of an October 2010, and of a late December 2010 PAFIC trap bee abundance (see Chapter 4) to add credibility to this conclusion. I then use the same bee data's spatial richness distributions to present a credible indication of the PAFIC trap's capacity for repeatable and consistent bee sampling data across varying flower environments.

## Methods

*PAFIC trap description and development*

The PAFIC trap used in this study is a combination of a traditional pan trap and a flight intercept trap (Samways *et al.* 2010). A size A4 sheet (210 mm X 297 mm) of clear Perspex (poly(methyl 2-methylpropenoate)) (Maizey (Pty) Ltd, Cape Town, South Africa) is used as a window to produce a clear barrier that intercepts the insect in flight. Two deep V-shaped notches (3 mm wide X 30 mm) are cut into opposite sides of a deep plastic pan of dimensions 25 cm diameter (at top) X 15 cm deep. The Perspex is pushed firmly into the notches so that the plastic sides grip it, with the Perspex extending approximately 20 cm above the top of the pan (Figure 5.1). I did not use adhesive to hold the Perspex in place as trapping was done only for 24-hour periods during windless or calm weather. The pan was filled with about 300 ml of water and a few drops (2 or 3) of a household biodegradable dish detergent were added to the water to reduce surface tension and facilitate insect trapping in the water.

(Figure 5.1)

*Theory of operation*

Bees caught in a traditional pan trap would either be attracted by the pan and fly directly into the dilute soap solution, or fly into the inside of the pan trap, collide with or land on the trap's smooth, inner, vertical plastic surface and then enter the dilute soap solution. Extending on this, it has been hypothesized that bees might simply examine the pan and not "visit" (enter into the volume enclosed by the pan's dimensions) in favour of more flower-like prospects (Cane *et al.* 2000). To catch such discriminating bee foragers, combining the pan with a vertical sheet of clear Perspex above it to combine a flight-intercept element to the trap would seem a logical next step.

The dependence of flight-intercept sampling consistency on insect flight direction consistency is debatable as is the effect of which direction the Perspex faces when positioning the trap. In this regard, significant research has been done on insect migration indicating strong directionality of migrational flight paths in certain species (Brown 1970, Chapman *et al.* 2003, Williams 1949) and on the homing, landmark recognition, and landmark imaging of many insects (Collett 1992 and references therein, Southwick & Buchmann 1995).

Many studies and reviews (e.g. Chittka *et al.* 2014, Collett 1992, de Ibarra *et al.* 2014, Giurfa 2007, Osborne *et al.* 2013, Srinivasan 2010) show quite clearly that bees (and other insects as well) have good capacity to visually analyze landmark elements and use them and images of them to efficiently find their

nests and to forage for nectar and pollen (and other rewards) in remarkably sophisticated ways, and to do so efficiently and repeatedly in remarkably complex environments. Such directed flight behaviour is more visually based, with olfactory senses being important near the nest (Southwick & Buchmann 1995). The implication is that, should a particular area have the correct substrate (whether soil or plant) for a bee species' nests and another area have the appropriate foraging for such bees, nectar/pollen foraging of these bees would be highly directional between such areas (hence the term, "bee-line"). Perspex sheets placed perpendicular to such insects' flight paths are more likely to catch such flying insects whereas if parallel to such a flight path there is a higher likelihood of the bee bypassing the sheet without striking it.

Alternatively, bees could distribute their individual nests (if completely solitary and non-social) or nest "clumps" (if social or eusocial to some extent) homogeneously throughout areas with uniform soil properties (for ground nesting bees) or uniform nest-plant distributions (for plant nesting bees). If forage availability is similarly scattered uniformly over an area thus inhabited (homogeneously) by bees then foraging directionality should be more random/non-directional in nature in this area. In this case the direction of the Perspex could theoretically be random with no significant effect from such bees' flight directionality on flight-intercept trap sampling.

This actual situation is rarely known before research in many (perhaps most) situations, so to be safe, flight-intercept trap users generally assume some insects' foraging flights are directional. To allow for consistent catches of directionally flying insect foragers flight intercept traps are placed facing one direction and an equal number of traps are placed perpendicular to this first direction (Samways *et al.* 2010). Alternatively the flight-intercept trap can be done for two consecutive days (provided weather, and other relevant conditions are consistent) and simply rotated by 90º after the first sampling day. In this way any biases for or against insects flying in certain directions is balanced.

*Evaluation*

Study area and methods

This study was conducted at Zachariashoek Valley (33.8291º S, 19.0518º E), Hawequas Nature Reserve (part of the larger Limietberg Nature Reserve), Western Cape, South Africa (Figure 3.1). This area was partially afforested with a managed pine plantation and was partially maintained as a pine-free, nature reserve. The entire area, pine trees and natural vegetation (fynbos) was burned in 1999. After the fire, the afforested area was not replanted with pines and the whole area was managed as a nature reserve with re-growing pines actively removed.

Two adjacent sub-sites, one which had consisted of natural fynbos prior to the fire (referred to as the Natural area) and one in the Previously Pine Recovery area (referred to as the PPR area), were selected for

comparison of the traditional pan trap and of the PAFIC trap. Nine of each kind of trap (PAFIC or traditional pan) were placed 60 m or further from the boundary between the Natural and Post-pine sub-sites in the general vicinity of whatever flowers were in bloom. Traditional and PAFIC traps were placed in visual approximation of the centroid of sparse patches of flowers provided that none of them were closer than 1 m. If the nearest flower from such a centroid was <1m then the trap was moved away from the centroid by the minimum amount that would still keep the nearest flower at least 1 m away. This was done for two consecutive 24-hour periods in the early/mid-spring of 2010 (end of September) during which the weather was cloud free and calm. The nine traditional pan traps were placed on the Natural side for the first 24-hour period. In the second 24-hour period the nine PAFIC pan traps were placed on the PPR sub-site (with the Perspex sheets directed randomly). The Natural vs. PPR difference of the experimental treatment (PAFIC vs. Traditional pan traps) might seem to confound this study but this is discussed below in the context of bee abundance differences ascertained in the Natural and PPR areas from a late December 2010 study. For both PAFIC and traditional pan trap types, three were primary blue, three were white, and three were bright yellow. After each 24 hour period, the traps were emptied using a strainer and the collected insects were stored in bottles with 70% ethanol. Bees were removed from these bottles, sorted into morphospecies and counted.

To ascertain if the PAFIC trap samples can overcome or even use the presence of a mass-flowering species, 13 PAFIC traps (seven yellow, three white, and three blue) were placed in both Natural and PPR sub-sites (for an aggregate of 26 traps) for two consecutive 24-hour periods on each sub-site during October of 2010. These 26 traps were placed with the pan in immediately adjacent and just touching a mass-flowering generalist pollination syndrome bush, *Agathosma capensis* (L.) Dummer (Rutaceae). The flat side of the Perspex of these PAFIC traps was directed to the centre of the flowering bush (Figure 5.2 (i)). These results were then compared to those of 65 PAFIC traps (35 yellow, 15 white, and 15 blue) placed in Natural and PPR sub-sites (for an aggregate of 130 traps) at the centroid of flower patches (Figure 5.2 (ii)) (in the same way mentioned as the pilot study above) for two days in late December of 2010 (the comparison-confounding issue of temporal change between October data and December data is discussed below). Although there were *A. capensis* bushes that were blooming in late December 2010 such bushes were sparse, had few flowers.

(Figure 5.2)

The bees caught during September, October, and December were all sorted carefully into morphospecies, counted, and voucher specimens of each morphospecies were sent for expert identification.

**Results and Discussion**

When comparing PAFIC vs. traditional pan traps directly, the nine PAFIC traps caught three times as many bees as the nine traditional pan traps (n=15 vs. n=5) which suggests that PAFIC traps are more effective, although repetition of this experiment would be necessary in order to be certain. Furthermore, repetition of this experiment should ensure that PAFIC and pan traps are compared simultaneously in adjacent areas which are expected to have identical bee assemblages.

This conclusion is strengthened in light of the PAFIC traps' higher number of bees being on the PPR side – the side that was shown to have significantly lower bee abundance (Wilcoxon's t =1029, P=0.0038) than Natural sub-sites (see Chapter 4 - Natural sub-sites had almost twice as many bees (n=333) as PPR sub-sites (n=181)). Temporal differences (September vs. December) and methodological ones (compositionally differing plant species assemblages) are confounding factors that prevent these two studies from being directly compared. However, only two species (*Lasioglossum* sp. 17, and the *Xylocopa* sp.) out of 11 that were compared in the December study had higher abundance in PPR sub-sites (and for those two, only one individual had been caught making these species' data not statistically analysable). This strongly indicates that the pattern of higher abundance in Natural sub-sites is a general rule for most bee species, including the ones from the September pilot study. This, in turn, strongly suggests that the triple number of bees (relative to pan trap) caught by the PAFIC trap in the PPR sub-site in the September pilot study is due to a higher PAFIC trap efficacy in catching bees.

Cane *et al.* (2000) mention that the presence of many blooming flowers has a potential inverse effect on the effectiveness of pan traps: the more flowers that bloom the less attractive the pan trap and the fewer insect flower visitors it catches. In my test, the PAFIC traps which were placed adjacent to mass-blooming *A. capensis* bushes in October 2010 caught similar proportions of bee species as the PAFIC traps placed in comparatively sparser flower patches in December 2010. The numbers of bee species unique to each of the Natural and the PPR sub-sites during September and October 2010 were the same (seven species unique to each side). This was in spite of a somewhat more dense growth of *A. capensis* plants in the PPR sub-sites. This Natural to PPR 1:1 ratio of unique species is nearly the same result as my other PAFIC trap study (see Chapter 4, Sampling Sessions 6 and 7) in which the PAFIC traps were not placed immediately beside any blooming flowers and a Natural to PPR ratio of 4:5 unique species was obtained. This consistent ratio indicates that my PAFIC trap can function independently of flowering presence by appropriate placement of PAFIC traps near pollinator attracting flowers. If flowers are sparse then the PAFIC trap should be placed in the general vicinity of flowers, and if they are concentrated/mass-flowering then the PAFIC trap should be placed immediately adjacent to them with the Perspex facing toward the centre of flower concentrations.

Table 5.1 shows that for the 12 species that were caught in both studies, eight of the species' Natural vs. PPR sub-site absence/presence data are in agreement, three are in partial agreement (the December PAFIC sampling had these on both sides whereas the October PAFIC sampling had these on the Natural side

only), and one (*Spinanthidium callescens* (Cockerell)) is in disagreement (caught on opposite sides). This demonstrates that PAFIC trap sampling yields consistent results even despite temporal sampling differences and the presence or absence of mass-flowering/florally dense displays.

(Table 5.1)

When assessing the sampling effort, it may be a preferable practice to place PAFIC traps immediately adjacent to flowers wherever there are concentrated, mass-flowering, or otherwise dominant floral displays, especially if the intention is to catch maximum number of species possible. This placement caught more species (27 bee species) than the study in which PAFIC traps were placed in the general vicinity of blooming flowers (25 bee species) despite employing only one fifth of the latter study's sampling effort (13 PAFIC traps in Natural and PPR sub-sites (for a total of 26 PAFIC traps) compared to 65 PAFIC traps in Natural and PPR sub-sites (for a total of 130 PAFIC traps)). It is possible, however, that the timing of the October PAFIC sampling, in the middle of the blooming season when insect flower visitation is normally at its highest, is the main reason for this sampling session's higher bee sampling efficacy.

Comparing averages of bee abundances over the two days per trap between the October (mass-flowering) PAFIC sample and the December (sparse flowering) PAFIC sample produces a slightly counter-intuitive result: while the adjacent October Mass-flowering PAFIC trap placement is more efficient at catching more species of bees (either due to season, or trap placement, or both – discussed below), the vicinity of Sparse-flowering PAFIC trap placement catches similar/slightly higher bee abundances per trap per day (Figure 5.3).

(Figure 5.3)

The explanation of this requires a proper experiment(s) designed specifically to address the question of why the October Mass-flowering PAFIC trap placement did not have the expected higher bee abundances on account of October being in the middle of the fynbos blooming season when more bees are typically active. Discovering the actual functioning of the PAFIC trap requires a proper experiment(s) designed with appropriate controls and with comparative trap placement methodologies being tested simultaneously, perhaps with high-speed cameras, and with sufficient sampling replicates for statistical comparison.

However, although the counter-intuitive higher abundance of caught bees in December may be due to many (temporal, stochastic, etc.) factors, it should be considered that trap placement adjacent to the mass-flowering bush may decrease the Perspex' effective flight intercept area in two ways. Firstly, assuming the *A. capensis* bush is the main attraction (by implication transforming the PAFIC trap to more of a Flight-Intercept trap than a Pan trap), the ratio of the Perspex collision area to the bush area decreases and the viable likelihood of the anthophiles colliding with the Perspex also decreases for a given level of "main attraction visitation" (compare available pathways of a bee towards the bush with available pathways towards the PAFIC trap in Figure 5.4 (i)). This mode of decreasing the PAFIC trap's catch numbers

becomes greater as the size of the bush or density of bushes in the region increases. Secondly, assuming the pan of the PAFIC trap is the main attraction in the adjacent-to-mass-flowering placement (in the centre-of-sparse-placement it is presumed to always be the main attraction) then, too, the available collision area of the Flight-Intercept element of the PAFIC trap is decreased as approach to the trap from one side is hindered by the larger *A. capensis* bush (compare available pathways of a bee towards the PAFIC trap adjacent to an *A.capensis* bush in Figure 5.4 (i) with available pathways of a bee towards the PAFIC trap in the vicinity of sparser flower patches in Figure 5.4 (ii)). Ultimately, even if the PAFIC trap is always the main attraction – whether adjacent to mass-flowering species or in the centroid of sparse flower patches – the centre-of-sparse-flower-patch placement implies more opportunity for effectiveness of the Flight-Intercept element of the PAFIC trap.

(Figure 5.4)

Traditional flight-intercept traps require a periodic rotation by 90º to remove the biasing effects of insects flying consistently in a certain direction. This labour-intensive modification is much facilitated with a simple 90º rotation of the PAFIC trap. The PAFIC trap direction is held with a wedge between the plastic pipe and the iron post and is simply rotated 90º on this support and the wedge re-inserted.

The rotation of the PAFIC trap when the trap is placed immediately beside large blooming floral displays is possibly unnecessary as insects would, theoretically, come towards the flower from various directions and, as long as the Perspex's flat side is facing toward the center of the floral display, a representative sample of such visitors should be obtained. However, to be rigorous in following correct flight-intercept methodology, this rotation of the Perspex by 90º could be effected by repositioning the whole pan trap to the right or left of its initial position and rotating the Perspex by 90º. Due to the rugged, rocky, and steep terrain, my PAFIC traps often had to be positioned with cable-ties and wooden struts attached to surrounding vegetation to hold the PAFIC traps upright and firmly in place so this repositioning was not feasible for this study.

A suggested improvement might be to use a bowl-shaped, scratch-resistant, clear pan (perhaps of glass) with a painted circle or even specific painted flower(s) to attract the bees/insects (Figure 5.5). The solid-colour, truncated cone of the traditional pan trap (Figure 5.1) might, in insect optical neuro-processing, have little resemblance to anything it would normally find in its natural environment. Alternatively, a much smaller (more typically flower-sized) pan could be used.

(Figure 5.5)

Due to the short periods of my testing of the PAFIC trap (two 24-hour periods), and due to the calm weather during which it was tested, no adhesive was used to affix the Perspex to the pan and only a simple wedge was used to keep the Perspex's direction. However, if the trap is used for longer periods of time or in

slightly windier conditions, adhesive may be required to keep the Perspex in place and a hole can be drilled through the pipe and the iron bar to allow the PAFIC trap to be pinned firmly to prevent rotation.

**Conclusion**

Much has been published on the efficacy of traditional pan traps for catching flower-visiting insects (Campbell & Hanula 2007, Cane *et al.* 2000, Munyuli 2013, Nielsen *et al.* 2011, Tuell & Isaacs 2009, Vrdoljak 2010, Westphal *et al.* 2008). However, my experience, though limited in scale, strongly indicates a good possibility, but does not rigorously prove, that the PAFIC trap is more effective for sampling bees. Also, it appears to be independent of surrounding floral displays if positioned appropriately close to such displays to catch anthophiles. These results are circumstantial evidence that surfaced from a larger study of bee species abundance and bee species richness and were not actually part of the larger objectives of those studies. However, while not statistically rigorous, this evidence demonstrates that the PAFIC trap holds promise compared to traditional methods and deserves further well-designed research to prove or disprove its effectiveness with traditional pan traps.

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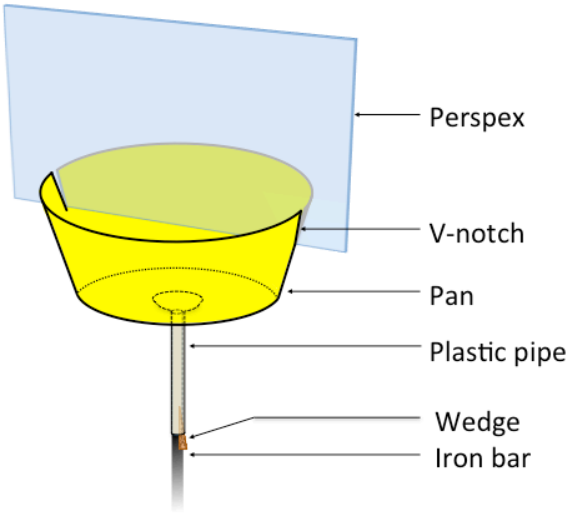


Figure 5. 1 Diagram of the Pan and Flight-Intercept Combination (PAFIC) trap.

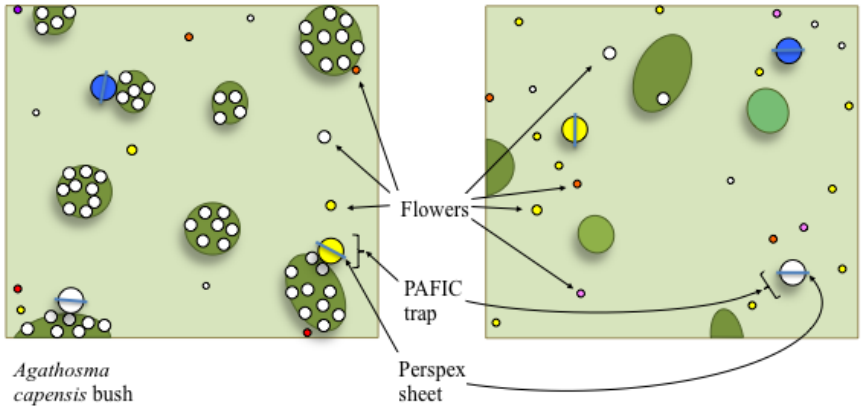


Figure 5. 2 Two placement methods for Pan and Flight Intercept Combination (PAFIC) trap use: (i) placement near a mass-flowering bush, *Agathosma capensis*, (ii) placement in general vicinity of centroids of sparser flower patches or high-density-flowers or mass-flowering species are present. Diagram not to scale.

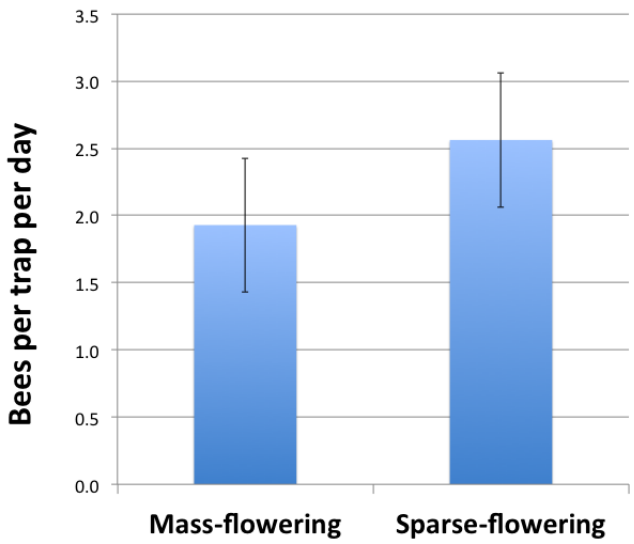


Figure 5. 3 Bee abundance (bees per trap per day) for Pan and Flight Intercept Combination (PAFIC) traps placed adjacent to mass-flowering plants compared to PAFIC traps placed in vicinity of sparse flowering plants (± one standard error) (data for Natural sub-sites only).

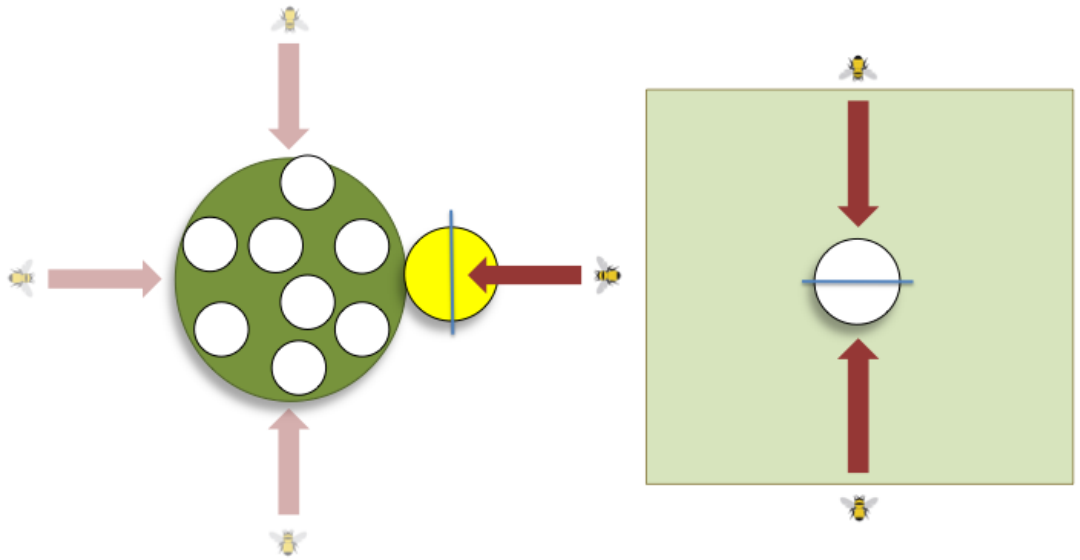


Figure 5. 4 Schematic diagram of approach paths to Pan and Flight Intercept Combination (PAFIC) traps: (i) immediate adjacency to an *Agothosma capensis* bush implies less effectiveness of Flight-Intercept element of PAFIC trap if the bush is the "main attraction" (faded arrows indicate other bee approaches that "escape" the PAFIC trap); (ii) when away from immediate adjacency of dense flower clumps (implying that bees caught in it perceive the PAFIC trap's pan colour as the "main attraction") there are still more approaches to the PAFIC trap than when the trap is impeded by the bush as in (i).

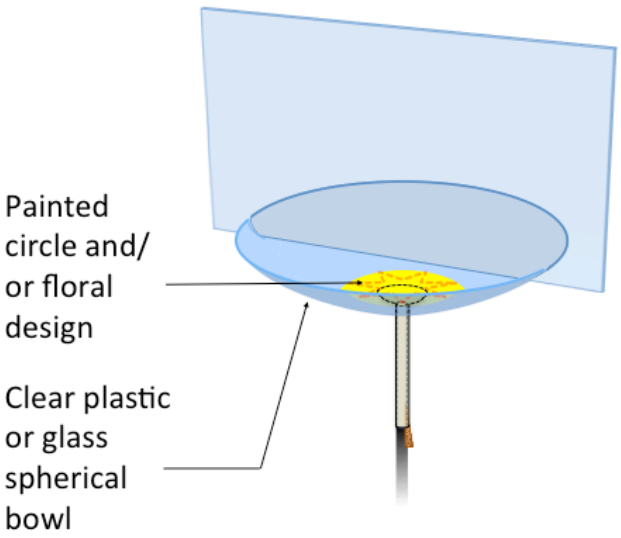


Figure 5. 5 A suggested alternative form of the Pan and Flight Intercept Combination (PAFIC) trap (Figure 5.1) of this study.

Table 5. 1 Species common to both Pant and Flight Intercept Combination (PAFIC) trap samplings of October 2010 (*Agathosma capensis* – mass-flowering) and December 2010 (sparse flowering). Spatial consistency of Absence/Presence of most species across Natural and Post-Pine Recovery (PPR) zones demonstrates PAFIC trap sampling spatial reliability despite different trap placement strategies of the mass-flower adjacency vs. sparser-flower vicinity methods of either study.

| Species common to both samples | October 2010 (mass-flowering) | | December 2010 (sparse-flowering) | |
|---|---|---|---|---|
| | Natural | PPR | Natural | PPR |
| *Xylocopa rufitarsis* Lepeletier | **Absent** | Present | **Absent** | Present |
| *Apis mellifera* Linnaeus | Present | Present | Present | Present |
| *Amegilla spilostoma* (Cameron) | Present | Present | Present | Present |
| *Ceratina aliceae* Cockerell | **Absent** | Present | Present | Present |
| *Spinanthidium callescens* (Cockerell) | **Absent** | Present | Present | **Absent** |
| *Lasioglossum* sp. 1 | Present | Present | Present | Present |
| *Lasioglossum* sp. 4 | Present | Present | Present | Present |
| *Lasioglossum* sp. 15 | Present | **Absent** | Present | Present |
| *Halictus (Seladonia)* sp. 1 | Present | Present | Present | Present |
| *Halictus (Seladonia)* sp. 2 | Present | Present | Present | Present |
| *Nomioides* sp. 1 | Present | **Absent** | Present | Present |
| *Patellapis* sp. 3 | Present | Present | Present | Present |

# Chapter 6 – Synthesis of vegetation and bee data and management recommendations

*Abundance patterns of plants and bees*

Plant species in general for sizes A (species that were never locally taller than 40 cm) and B (species with maximum height between 40 and 140 cm) together or separate showed that the Natural sub-sites had higher plant abundance than the PPR sub-sites for Deep and Ecotone data combined. Significant differences were found for about half of the species in the study with two thirds of these statistically higher in abundance in the Natural sub-sites. Only one third of these were statistically more abundant on the PPR sub-sites. Plant groups that matched these patterns included *Erica* spp., *Restio* spp., and *Hypodiscus* spp. which were more abundant in the Natural sub-sites while *Helichrysum* spp. were more abundant in the PPR sub-sites.

All bee species, genera, families, and complexes had higher abundance in the Natural sub-sites with the exception of one complex – *Lasioglossum* complex 1. Of these, all species, genera, families, and complexes with enough bees for statistical analysis and that did show a statistical difference between Natural and PPR, confirmed this same conclusion, being statistically more abundant in Natural than in PPR sub-sites. As the study was mainly designed for generalist bees, and bee species in this study were mostly from generalist oligolectic genera as verified by specialist observation, this trend of lower bee abundance in PPR areas applies to this generalist category of bees. However, as many specialist pollination syndrome plants (Johnson 2010), although not all (Johnson & Steiner 2000), are visited by generalist pollinators the lower levels of generalist bees is indicated as an "alarm-bell" for pollination limitation for plants on the whole, including many specialist plant species, in PPR areas.

The general correspondence of lower plant abundance to lower bee abundance is attributable to the disturbance affected by lengthy pine shading and litterfall followed by a hotter-than-usual pine forest fire (discussed below).

*Richness patterns of plants and bees*

Natural sub-sites had higher total plant species richness than PPR sub-sites (Natural: 126 species; PPR: 88 species; excluding geophyte-like species & unidentified species). This pattern differed from bees, for which spring and early summer PAFIC sampling demonstrated no significant difference in richness between Natural and PPR sub-sites (Spring sampling: Natural - 20 species, PPR – 20 species; Early summer sampling: Natural – 20 species, PPR – 21 species). This suggests that plant biodiversity is not necessarily a good surrogate for bee biodiversity.

*Chicken or egg?*

The causality of bee declines impeding plant recovery and/or plant species declines impeding the recovery of bees is a complex but interesting issue and raises the classic "chicken or egg" question common in the life and social sciences. Was disturbed soil the reason that bees could not nest, and as a result, plants reliant on their pollination could not recover? Or was the lack of recovery of plants and concomitant deficient pollen and nectar nutrition for bees the reason that bees could not recover? The complexity is made more intricate by the degree of interaction with abiotic factors such as changes in soil. Figure 6.1 illustrates in a simplistic way these major role-players and their interactions. Ultimately these elements form a combined interaction web that is influenced by the synergy of the three major disturbances of pine shading, pine litterfall, and hot pine forest fire in Zachariashoek.

(Figure 6.1)

*Implications for pollination-webs*

Kearns *et al.* (1998) mentions that pollination "interaction webs" are richly connected in general and exhibit significant temporal and spatial variation. The potential possibilities for pollination webs seem quite broad given the highly speciose fynbos flora that, while outwardly very similar to botanically uninitiated humans, may actually be quite "obviously distinguishable" from the bees' perspectives. If the general conclusion that bees are lower in number in PPR areas holds true for other disturbed areas in the CFR, then in these disturbed areas, there will simply not be enough bees to fill such pollination "webspaces", however rich and complex such interaction networks could potentially be. On the whole, however, the plant species richness on PPR sub-sites was lower indicating that pollination webs for common plant species would be significantly simpler than those in Natural sub-sites.

The pollination limitation is likely applicable to some degree in other insect flower visitors in other insect families. This means that pollination limitation is a hurdle for the recovery of indigenous flora in such areas and that pollination as a keystone process needs to be a focus in restoration strategies for such areas.

*Taxonomic knowledge gap(s)*

A total of 37 (possibly up to 44 including unique males as separate species) species were undescribed in this study. These were meticulously resolved in the laboratory to a degree that even phenotypic variations within more numerous species were noted and initially sorted accordingly. Bees with such variations were initially kept separate; they were pooled to the same species only with expert

confirmation that these variations were from the same species. In only two cases were bees assigned to species complexes (*Lasioglossum* complexes 1 and 2) due to difficulty in species resolution.

The high number of undescribed species, coming only from one valley, demonstrates how little is known of apifaunal diversity in the CFR biodiversity hotspot and this taxonomic knowledge gap is probably extendable to other similar areas of high biodiversity. It has already been suggested that the family Halictidae need to be revised (Connal Eardley, personal communication). These taxonomic knowledge gaps need to be overcome in order to correctly describe pollination networks in such biodiverse areas for development of management strategies to enhance recovery and restoration of keystone processes.

*Issues of scale*

This number of undescribed species comes in the backdrop of another surprising figure: the total number of bees present in Zachariashoek was 56. Based on the missing species, one would normally expect in the Apidae, Megachilidae, and Colletidae, and the temporally and topographically incomplete nature of the study, the true number of species is more correctly estimated at 100 for the Zachariashoek valley (Connal Eardley, personal communication). For comparative purposes, a recent study of Mediterranean areas where bee diversity is normally considered to be the highest globally (Michener 2007), the highest number of species for a particular area, olive groves, was 50 (Potts *et al.* 2006).

The 100 species in the Zachariashoek valley (which had an area of 3.5 $km^2$) translates to 28.5 species per $km^2$. On the smaller scale of the 56 confirmed species for the total area of sampling in the five sites (approximately 6.7 ha total) this figure changes to 8.3 species per ha. Converting the 100 species in the 3.5 $km^2$ valley to species per ha, the figure changes to 0.29 species per ha or conversely taking the 8.3 species per ha and calculating for the valley a figure of 830 species per $km^2$ is arrived at assuming complete turnover. The obvious lack of correlation to "on-the-ground" reality of the numbers 0.29 species per ha, which assumes no interspecies area overlap, or 830 species per $km^2$, which assumes complete turnover, demonstrates the necessity of correct useage of scale when reporting species per unit area values.

The more meaningful figures of 28.5 species per $km^2$ or 8.3 species per ha are probably low. This is because they are not temporally representative over a complete year as I only IBS net sampled in Autumn and only PAFIC trap sampled for four days in one site only in spring and two days in early summer. As well the figures are not spatially representative as I only sampled in generally lower fynbos mountain slopes and did not sample in other important and often extremely biodiverse habitat zones such as riparian zones, mountain tops, or fynbos valley basins. Given fynbos' famously high plant species turnover (**β**-diversity) such areas might come with many other unique bee species.

*Future prospects in Zachariashoek and lessons learned*

The data from this study are only a "snapshot" for 13-year old fynbos. It would be interesting to see the dynamics in the recovery of fynbos after, for example, 26 years and at 52 years. Long-term results like this would give a truer picture about recovery of bee populations given insect notoriety for stochastic temporal variation (Cane & Payne 1993, Williams *et al.* 2001). This is especially true in a frequent fire adjusted ecosystem like fynbos where post fire successional stage has a remarkable effect on the plant community composition and in which insects are probably uniquely adapted to take best advantage of such frequent vegetation change.

Much has been published on the breakdown and restoration of pollination webs (Forup & Memmott 2005, Memmott 1999, Memmott & Waser 2002, Pauw 2007) but such knowledge would need reinterpretation in fynbos pollination systems in the light of more correct estimates of the number of undescribed and unknown bees in fynbos. With this in mind, what is the answer to the question of how bee pollination webs can be restored in post pine-afforested areas? Given that 11 years of passive management still shows significant bee pollinator recovery delay and a substantially impoverished flora it is suggested here that active restoration means be attempted to improve bee and other insect flower visitor and plant recovery.

This procedure is in line with published restoration suggestions that fynbos plant species need to be replanted/reseeded immediately after fire to engender the maximum recovery of natural fynbos at the fastest rate (Holmes & Richardson 2002). There has been success at restoring areas after pine tree afforestation, provided that seed is spread with soil that has been in some way treated with heat and smoke (i.e. the effects of fire – *sensu* Holmes (2001) and that this is done *immediately* after the clearing of the pine trees, before other vegetation has moved in. Holmes (2001) reports some success in establishing Proteoid fynbos with this method.

In the case where no such treatment is given and disturbance adapted vegetation is given freedom to make whatever headway it can into the abnormal *P. radiata*-disturbed soil, the problem becomes somewhat complex. On the one hand the disturbance adapted vegetation is an improvement over the pine trees. Indeed, in Zachariashoek arguably there was an 8 800 % improvement, given the 88 new species growing there that were not there under the pines. On the other hand, these 88 species are compositionally quite different from the species in the Natural sub-site. This difference increases in deeper regions of the PPR sub-site where 16 out of 17 species recorded there were *not* recorded at all in the common plants on the Natural sub-site.

The PPR sub-sites show a very obvious level of soil disturbance. My study suggests that *Metalasia densa*, *Stoebe plumosa*, and *Stoebe cinerea*, have a high success rate in recolonizing the PPR areas, indicating these species as disturbance-adapted. The influx of pioneering disturbance-adapted species is a commonly observed ecological phenomenon and such plant species are categorized as ruderal plants with

degree of ruderality and its measurement a topic and tool of research itself (Grime 1979, 2001, Hill *et al.* 2002, Pywell *et al.* 2003). The global phenomenon of ruderal plant species' success in disturbed areas contrasts to the dubious success rates of other higher conservation value plant species reintroduction measures (Godefroid *et al.* 2011, Heelemann *et al.* 2012, Li *et al.* 2012). There is a need to look at other measures of improving CFR restoration measures to establish plant species of higher conservation value.

The botanical focus that restoration measures usually take may stand to benefit from an insect's perspective in order to be more holistic and ecosystem-process focused. In this regard, an important question arises: how does one encourage normal insect assemblages' recovery/reinstatement in PPR areas? Such recovery may help normal plant community recovery as such insects are important in many ecosystem processes. The first to inhabit PPR areas are presumably insects that fly, and for this reason the part that bees play in soil should not be overlooked, especially as most bees make their homes in the soil (Michener 1964).

Bees play an important role in the maintenance of soil macroporosity and the ability of soils to absorb water as a result (Cardale 1968, Houston 1969, Knerer & Schwarz 1976). The encouragement of such recovery has usually been from the perspective of the importance of bees in agro-ecological systems, with a strong focus on the bee species, usually honeybees, that are specifically important for agriculture (e.g. Eardley *et al.* 2006).

Short-term restoration goals normally attempt to restore "as much life" as possible. Such a perspective overlooks the finding that disturbance-adapted bees, unique to or associated with PPR and other disturbed areas, may actually out-compete other pristine-environment-adapted bee species or insect pollinators, and decrease them to some extent in recolonizing an area. Such pioneering bee species, although indigenous, may play an ecological role in the same way that recently naturalized honeybees (*Apis mellifera*) and one invasive bumblebee (*Bombus terrestris*) are already doing in parts of the world. Both of these bee species have been shown to increase seed set in weedy plant species (Stout *et al.* 2002) and to cause some displacement of native apifauna (Roubik 1996).

In this regard *Lasioglossum* species, often reported from disturbed areas (Connal Eardley, personal communication) might seem to indicate that the bees from the entire area of study were from "disturbed areas." However, as all of the *Lasioglossum* species, except two, were more abundant in the Natural sub-sites, this genus, though common in disturbed areas, is indicated as also an integral part of pristine landscapes, and as such an important part of natural pollination networks.

**Management suggestions**

In conservation, a hierearchy of prioritization can be simplified as follows: (1) preserve and protect as much heterogenous and biodiverse natural areas as possible; (2) mitigation and conservation-conscious management of agriculture and land-transforming development; and (3) restoration as a last step for reversing human interventions in natural areas (Hobbs & Harris 2001, Samways 2005, Samways *et al.* 2010).

Restoration should ultimately be holistic and look to the benefit of all aspects of natural ecosystems – including the inescapable human dimension(s) thereof. Reductionist science in this regard may be lacking in "wisdom" as it looks only at parts of ecosystems and not at the whole (Heller & Zavaleta 2009).

Taking this into consideration, the restoration recommendations and management suggestions for the return of normal bee and vegetation biodiversity in previously pine-afforested areas, as mentioned here, need to be taken in the cautionary and tentative spirit with which they are submitted.

In this regard, it might be helpful to consider efforts at the restoration of ecological keystone *processes* such as pollination and invertebrate-mediated processes for soil health (*sensu* Colloff *et al.* 2010 and references therein). This could be accomplished by one of, or any combination of, six methods presented here not necessarily in order of preference: (1) do nothing; (2) find and restimulate surviving fynbos soil and fynbos soil seedbank; (3) perform soil "transplants" from natural areas; (4) perform fynbos wood and above-soil plant residue transplants; (5) perform revegetation measures with wind-pollinated indigenous fynbos families; and (6) remove excessive dead pine trunks. These are explained in detail as follows:

(1) Do Nothing. Pedogenesis has its natural way and a return to healthy soils and healthy soil ecosystem processes is an inevitable occurance – although this might take decades or centuries, or even millenia (Targulian & Krasilnikov 2007).

(2) Find and restimulate surviving fynbos soil and fynbos soil seedbank (Esler *et al*. 2014). Versfeld (1981), as reported by Richardson & VanWilgen (1986), states that 372 g/m$^2$/yr is the amount of litterfall for 35-year old stands of *P. radiata* compared to 217 g/m$^2$/yr litterfall for 22-year old stands of mountain fynbos. This means that viable fynbos seeds in buried seed banks may actually be surviving under layers of *P. radiata*-disturbed soil. This is a hopeful option especially since seed in seedbank has been shown to be viable even after longer terms of dormancy for fynbos (Holmes & Cowling 1997a, Holmes & Cowling 1997b) and for other CFR vegetation types (Heelemann *et al.* 2013). The proposition is that careful, controlled removal of such surface layers of abnormal soil, stopping once original fynbos soil is reached, followed by very controlled microburns (singeing) on such excavated areas might stimulate surviving fynbos seed and soil to sprout. It would be crucial to stop at the right depth as the viable seed storing layer of fynbos topsoil is <100 mm and typically 40-50 mm thick (Putwain & Gillham 1990). This might force such efforts to be labour-intensive, possibly entirely manual and possibly

limited to a few small plots to act as catalysts for the renewal of fynbos vegetation and ecosystem processes. This is arguably a controversial suggestion, given that soil movement is normally perceived as soil erosion which, in the context of conservation, is normally presented as a barrier to restoration measures. A final alternative is to perform careful ploughing (as mentioned by Joubert *et al.* (2009) in Agulhas sandstone fynbos) of soil to expose fynbos seedbank followed by seed-sprouting stimuli such as smoke, mild singing, etc.

(3) "Transplant" soil from natural areas. This labour-intensive alternative would be an alternative to tree revegetation as mentioned by Colloff *et al.* 2010, particularly as fynbos has few, if any, trees in most of its areas or other plant reseeding and plant seedling revegetation measures should they fail, as is not uncommon (Godefroid *et al.* 2011, Heelemann *et al.* 2012). The aim is to translocate volumes of soil (*sensu* Holmes (2001) from nearby pristine areas into suitable excavated patches of disturbed soil in PPR zones. The excavated disturbed soil could be used to backfill the excavations in the pristine areas. Excavations of soil from pristine areas could perhaps even include small healthy termite mounds (if they do not extend very deeply). Such translocation should ensure that no vulnerable endangered plants are disturbed. Such patches of healthy soil refugia and hopefully any plant life that could survive the translocation might become continual sources of normal fynbos seed propogation and might also act as catalysts to hasten the re-activation of arthropod-mediated soil ecosystem processes, pedogenesis, and other ecological keystone processes. Such "transplant" patches might do better with some initial watering as well to encourage recovery.

(4) Transplant normal fynbos dead wood and above-soil plant residue. Transplanting of dead wood and above-soil plant residue would encourage the recovery of organisms, arthropods, and other biota that inhabit such organic plant matter, provided such transplants does not harm threatened or endangered plants.

(5) Revegetate with wind-pollinated indigenous fynbos families. The wind-pollinated Restionaceae are a large group of plants that forms an integral and, due to perhaps its less "showy" nature, unfortunately over-looked part of the fynbos (Dorrat-Haaksma & Linder 2012) that would *not* suffer any pollination-limitatation as far as bee-pollinator limitation is directly concerned. My data on Restionaceae recovery are mixed, with some genera cautiously indicated as not doing well: *Hypodiscus* and *Restio*. However, one species from the 10 *Restio* species distinguished in our study was more abundant in PPR sub-sites (*Restio* (subgen. *Ischyrolepis*) sp.). Therefore, since the possibility of recovery does exist with passive recovery, there is greater potential with assisted restoration methods. Initial active restoration with Restionaceae has the short-term goal of "jump-starting" ecosystem process recovery in soil and the long-term goal of recovery of all plants, insects, and associated interactions and soil processes.

(6) Remove dead pine trunks in PPR areas. Some bees make their nests in the excavations of various wood-boring insects. The probability is that dead pine trunks, being relatively new to fynbos entomofauna, are only bored by a few pine-trunk adapted wood boring insects and this

may mean that only a narrow variety of bees can make homes therein. Should this be the case, then such bees may find post pine-afforested areas perfect for nest making, and this might create a distinctly lop-sided bee biodiversity in favour of such bees. This could lead to irregular and "weedier" bee assemblages. Additionally, the presence of such dry pine trunks as a significant fuel source close to the ground poses a more severely soil-disturbing fire hazard as fires in areas with felled tree trunks normally transfer much more heat to the soil (Cilliers *et al.* 2004, Holmes *et al.* 2000).

As for the CFR's more renowned endangered endemic plants, however, how long would complete recovery take? The recovery effort of Zachariashoek has certainly been a remarkable and positive step in the right direction as, prior to the 1999 fire that burned the forest and fynbos surrounding it, the area under the pines probably had very few, if any, of the 88 confirmed species that now reside there, a jump by *at least* 8 800% as there were other unidentifiable species and as this number excludes grasses and sedges.

Having spent many days inside managed mature pine tree forest stands, i.e., 20 year old stands, I can confirm that there are no insect flower visitors in there, although I did once see a *Xylocopa* sp. of bee come into the edge and then immediately fly straight up the canopy and above in search of more normal fynbos. So, in this sense, the 50 species of bees that I did confirm to be in the PPR sub-site also represents a 5 000 % jump in bee species richness in PPR areas. However, as far as fynbos plant species of higher conservation value are concerned, and with consideration also of bee species abundance, a 13-year period still leaves a long way to go for complete recovery. Some semblance of naturalness may only be achieved after several adequately spaced (15 years or longer) burns, perhaps taking 50 years or longer.

Ultimately all these measures are suggestions and only their application, time, and appropriate monitoring measures will tell if they truly have biodiversity restoration value.

*Final management suggestion regarding fire for restoration from pine afforestation*

The hot fires that can occur in pine afforested areas, especially if they are accidental and occur under very dry conditions, is potentially a severe disturbance to an area already disturbed by extensive pine shading and pine litterfall. The disturbance in Zachariashoek is not inevitable for all pine-afforested areas if the majority of the ecological disturbance in Zachariashoek came from the extreme heat of the fire as is proven for fynbos seedbanks after fires in stands of pine trees (Cilliers *et al.* 2004). Such a proposed larger proportion of disturbance being attributed to fire for bees as well, can only be proven by comparative studies of pine afforested areas restored to fynbos without a pine forest fire. In the absence of such studies, the precautionary principle points to "presume guilty till proven innocent", i.e., that accidental dry/hot season pine forest fires can contribute significantly to disturbances to bees as well in pine afforested areas.

With this understanding it is recommended that accidental fires under dry and/or windy conditions in pine afforested areas slated for restoration to indigenous vegetation be guarded against to prevent the possible disturbances that they may cause.

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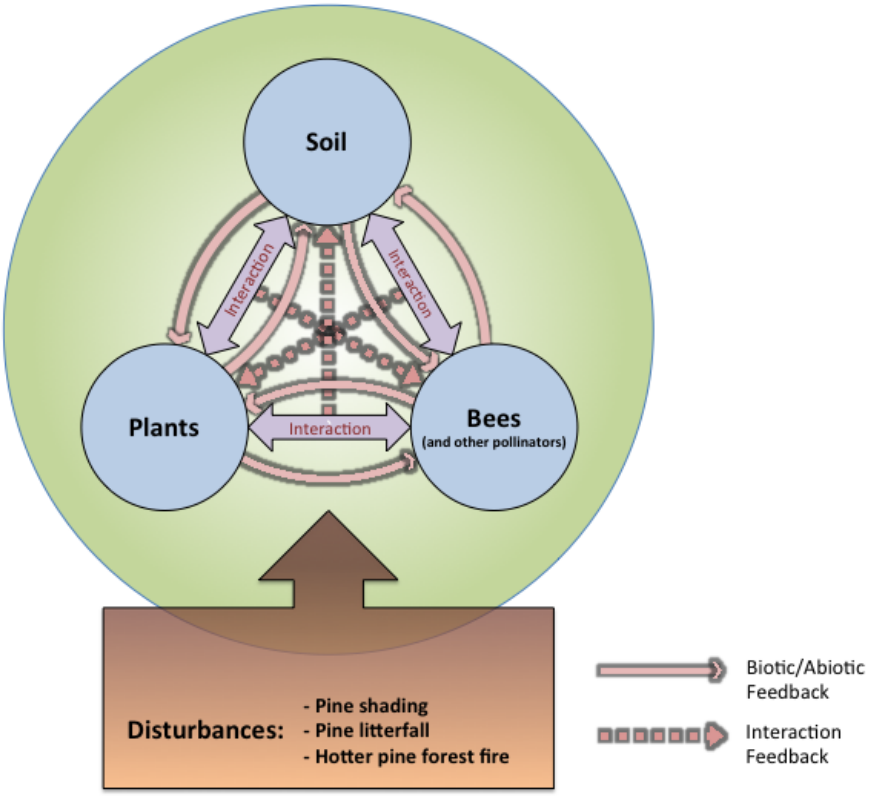


Figure 6. 1 Simplified diagram of ecosystem components and their interactions. In Zachariashoek these were disturbed as a whole by a synergy of pine shade, pine litterfall, and hotter pine forest fire. This diagram could of course include other components (air, mammals, water, etc.).

# Appendices

**Appendix 1 - List of all plants identified to species, genus, or family level.**

| Species no. | Family | Taxonomic level of identification |
|---|---|---|
| 1 | Aizoaceae | Aizoaceae sp. |
| 2 | Anacardiaceae | *Heeria argentea* (Thunb.) Meisn. |
| 3 | | *Searsia angustifolia* (L.) F. A. Barkley |
| 4 | | *Searsia dissecta* (Thunb.) Moffett |
| 5 | | *Searsia lucida* (L.) F. A. Barkley |
| 6 | | *Searsia rosmarinifolia* (Vahl) F. A. Barkley |
| 7 | | *Searsia tomentosa* (L.) F. A. Barkley |
| 8 | Apiaceae | *Centella villosa* L. |
| 9 | | *Peucedanum galbanum* (L.) Benth. & Hook. f. |
| 10 | Asparagaceae | *Asparagus lignosus* Burm. f. |
| 11 | | *Asparagus retrofractus* L. |
| 12 | | *Asparagus rubicundus* P. J. Bergius |
| 13 | Asteraceae | *Berkheya herbacea* (L. f.) Druce |
| 14 | | *Berkheya* sp. |
| 15 | | *Elytropappus glandulosus* Less. |
| 16 | | *Eriocephalus africanus var. paniculatus* (Cass.) |
| 17 | | *Euryops abrotanifolius* (L.) Moench |
| 18 | | *Felicia filifolia subsp. filifolia* (Vent.) Burtt Davy |
| 19 | | *Helichrysum asperum* (Thunb.) Hilliard & B.L. Burtt |
| 20 | | *Helichrysum* sp. * |
| 21 | | *Helichrysum cylindrifolium* (L.) Hilliard & B.L. Burtt |
| 22 | | *Helichrysum indicum* (L.) Grierson |
| 23 | | *Helichrysum teretifolium* (L.) D. Don |
| 24 | | *Helichrysum zeyheri* Less. |
| 25 | | *Metalasia densa* (Lam.) P. O. Karis |
| 26 | | *Osteospermum moniliferum* L. |
| 27 | | *Osteospermum* sp. (? *O. ciliatum*) |
| 28 | | *Othonna parviflora* B.J. Bergius |
| 29 | | *Phaenocoma prolifera* (L.) D. Don |
| 30 | | *Senecio burchellii* DC. |
| 31 | | *Senecio pubigerus* L. |
| 32 | | *Senecio rigidus* L. |

Appendix 1 continued

| Species | Family | Taxonomic level of identification |
|---|---|---|

| no. | | |
|---|---|---|
| 33 | | *Stoebe cinerea* (L.) Thunb |
| 34 | | *Stoebe plumosa* (L.) Thunb. |
| 35 | | *Syncarpha canescens* (L.) B. Nord |
| 36 | | *Ursinia paleacea* (L.) Moench |
| 37 | Boraginaceae | *Lobostemon* sp. |
| 38 | Brassicaceae | *Heliophila* sp. |
| 39 | Campanulaceae | *Prismatocarpus brevilobus* A. DC. |
| 40 | | *Wahlenbergia exilis* A. DC. |
| 41 | Celastraceae | *Maytenus oleoides* (Lam.) Loes. |
| 42 | Cyperaceae | *Ficinia bulbosa* (L.) Nees |
| 43 | | *Ficinia duesta* (P.J. Bergius) Levyns |
| 44 | | *Ficinia ecklonea* (Steud.) Nees |
| 45 | | *Ficinia indica* (Lam.) Pfeiffer |
| 46 | | *Ficinia levynsiae* T.H. Arnold & Gordon-Gray |
| 47 | | *Ficinia nigrescens* (Schrad.) J. Raynal |
| 48 | | *Ficinia oligantha* (Steud.) J. Raynal |
| 49 | | *Ficinia tristachya* (Rottb.) Nees |
| 50 | | *Tetraria cuspidata* (Rottb.) C. B. Clarke |
| 51 | | *Tetraria nigrovaginata* (Nees) C. B. Clarke |
| 52 | | *Tetraria picta* (Boeckeler) C. B. Clarke |
| 53 | | *Tetraria pubescens* Schonl & Turril |
| 54 | | *Tetraria sylvatica* (Nees) C. B. Clarke |
| 55 | | *Tetraria ustulata* (L.) C. B. Clarke |
| 56 | | *Tetraria variabilis* Levyns |
| 57 | Ebenaceae | *Diospyros glabra* (L.) DeWinter |
| 58 | Ericaceae | *Erica abietina* (L.) subsp. *aurantiaca* |
| 59 | | *Erica articularis* L. |
| 60 | | *Erica bicolor* Thunb. |
| 61 | | *Erica imbricata* L. |
| 62 | | *Erica nudiflora* L. |
| 63 | | *Erica plukenettii* L. subsp. |
| 64 | | *Erica rigidula* (N. E. Br.) E. G. H. Oliv. |
| 65 | Euphorbiaceae | *Clutia* sp. |
| 66 | Fabaceae | *Aspalathus cordata* (L.) R. Dahlgren |

Appendix 1 continued

| Species no. | Family | Taxonomic level of identification |
|---|---|---|
| 67 | | *Aspalathus crenata* (L.) R. Dahlgren |

| | | |
|---|---|---|
| 68 | | *Aspalathus* sp. 1 |
| 69 | | *Aspalathus* sp. 2 |
| 70 | | *Otholobium* sp. |
| 71 | | *Podalyria leipoldtii* L. Bolus |
| 72 | Haemodoraceae | *Dilatris* sp. |
| 73 | Iridaceae | *Moraea* sp. |
| 74 | Lamiaceae | *Salvia* sp. |
| 75 | Lauraceae | *Cassytha ciliolata* Nees |
| 76 | Linaceae | *Linum africanum* L. |
| 77 | Malvaceae | *Hermannia* sp. |
| 78 | Menispermaceae | *Cissampelos capensis* (L. f.) Diels |
| 79 | Molluginaceae | *Polpoda capensis* C. Presl |
| 80 | Montiniaceae | *Montinia caryophyllacea* Thunb. |
| 81 | Myrsinaceae/Primulaceae | *Myrsine africana* L. |
| 82 | Oleaceae | *Olea europa* subsp. *africana* |
| 83 | Orchidaceae | *Holothrix villosa* Lindl. |
| 84 | Poaceae | ? *Tenaxia* (*Merxmuellera*) *stricta* (Schrad.) Conert |
| 85 | | *Ehrharta calycina* Sm. |
| 86 | | *Ehrharta longifolia* Schrad. |
| 87 | | *Ehrharta ramosa* (Thunb.) Thunb. |
| 88 | | *Geochloa* (Merxmuellera) *rufa* (Nees) Conert |
| 89 | | *Pentameris* (Pentaschistis) *curvifolia* (Schrad.) Stapf. |
| 90 | | *Pentameris* (Pentaschistis) *eriostoma* (Nees) Stapf. |
| 91 | | *Pentameris* (Pentaschistis) *malouinensis* (Steud.) Clayton |
| 92 | | *Pentameris* (Pentaschistis) *pallida* (Thunb.) H.P.Linder |
| 93 | | *Themeda triandra* Forssk. |
| 94 | | *Tribolium uniolae* (L. f.) Renvoize |
| 95 | Polygalaceae | *Muraltia* cf. *hyssopifolia* |
| 96 | Proteaceae | *Brabejum stellatifolium* L. |
| 97 | | *Leucadendron rubrum* Burm. f. |
| 98 | | *Leucadendron salignum* P. J. Bergius |
| 99 | | *Leucadendron* sp. |
| 100 | | *Protea neriifolia* R. Br. |

Appendix 1 continued

| **Species no.** | **Family** | **Taxonomic level of identification** |
|---|---|---|
| 101 | | *Protea nitida* Mill. |
| 102 | | *Protea repens* (L.) L. |
| 103 | | *Protea scorzonerifolia* (Salisb. Ex Knight) Rycroft |

| | | |
|---|---|---|
| 104 | | *Protea* sp. 1 |
| 105 | | *Protea* sp. 2 |
| 106 | | *Serruria fasciflora* Salisb. Ex Knight |
| 107 | | *Serruria* sp. |
| 108 | Pteridaceae | Pteridaceae sp. 1 |
| 109 | | Pteridaceae sp. 2 |
| 110 | Ranunculaceae | *Anemone vesicatoria* Prantl |
| 111 | Restionaceae | *Askidiosperma sp.* |
| 112 | | *Elegia filacea* Mast. |
| 113 | | *Hypodiscus argenteus* (Thunb.) Mast. |
| 114 | | *Hypodiscus aristatus* (Thunb.) C. Krauss |
| 115 | | *Hypodiscus striatus* (Kunth.) Mast. |
| 116 | | *Hypodiscus willdenowia* (Nees) Mast. |
| 117 | | *Restio* (subg. *Ischyrolepis*) *capensis* (L.) H.R. Linder & C.R. Handy |
| 118 | | *Restio* (subg. *Ischyrolepis*) *gaudichaudiana* (Kunth.) H. P. Linder |
| 119 | | *Restio* (subg. *Ischyrolepis*) *monanthus* Manth. |
| 120 | | *Restio* (subg. *Ischyrolepis*) *sieberi* Kunth. |
| 121 | | *Restio* (subg. *Ischyrolepis*) sp. 1 |
| 122 | | *Restio* (subg. *Ischyrolepis*) sp. 2 |
| 123 | | *Restio* (subg. *Ischyrolepis*) *tenuissimus* Kunth. |
| 124 | | *Restio* (subg. *Locapsis*) sp. |
| 125 | | *Restio* (subg. *Restio*) *filiformis* Poir |
| 126 | | *Restio* (subg. *Restio*) sp. |
| 127 | | *Staberoha* sp. |
| 128 | | *Thamnochortus* sp. |
| 129 | | *Willdenowia glomerata* (Thunb.) H. P. Linder |
| 130 | Rhamnaceae | *Phylica pinea* Thunb. |
| 131 | | *Phylica strigulosa* Sond. |
| 132 | Rosaceae | *Cliffortia ruscifolia* L. |
| 133 | Rubiaceae | *Anthospermum aethiopicum* L. |
| 134 | | *Anthospermum capense* (L.) (??) |

Appendix 1 continued

| **Species no.** | **Family** | **Taxonomic level of identification** |
|---|---|---|
| 135 | Rutaceae | *Agathosma capensis* (L.) Dümmer |
| 136 | | *Agathosma* sp. |
| 137 | | *Diosma meyeriana* Spreng. |
| 138 | | Rutaceae sp. 1 |
| 139 | Santalaceae | *Thesium* sp. |

| | | |
|---|---|---|
| 140 | Scrophulariaceae | *Microdon dubius* (L.) Hilliard |
| 141 | | *Oftia africana* (L.) Bocq. |
| 142 | | *Pseudoselago spuria* (L.) Hilliard |
| 143 | Thymelaceae | Thymelaceae sp. |
| 144 | | *Passerina truncata* (Meisn.) Bredenkamp & A. E. vanWyk |
| 145 | | *Struthiola ciliata* (L.) Lam. |
| 146 | Xanthorrhoeaceae | *Bulbinella trinervis* (Baker) P. L. Perry |
| 147 | | *Caesia contorta* (L. f.) T. Durand & Schinz |
| 148 | Zygophyllaceae | *Zygophyllum* sp. |

**Appendix 2 - List of all plants identified as morphospecies:**

| Morphospecies Name | Comments/Description |
|---|---|
| Unknown sp 1 | Geophyte – like |
| Unknown sp 2 | Geophyte – like |
| Unknown sp 3 | Geophyte - like: *Gladiolus* sp. |
| Unknown sp 4 | Geophyte – like |
| Unknown sp 5 | Geophyte – like |
| Unknown sp 6 | Geophyte – like |
| Unknown sp 7 | Geophyte – like |
| Unknown sp 8 | Geophyte – like |
| Unknown sp 9 | Geophyte – like |
| Unknown sp 10 | Geophyte – like |
| Unknown sp 11 | Geophyte – like |
| Unknown sp 12 | Geophyte – like |
| Unknown sp 13 | Geophyte – like |
| Unknown sp 14 | ? *Pelargonium* sp. |
| Unknown sp 15 | ? *Pelargonium* sp. |
| Unknown sp 16 | tough long sprawling restioid culm; impossible to pull out with roots/bulb |
| Unknown sp 17 | Ericoid; black glands; ? Rutaceae |
| Unknown sp 18 | Ericoid; black glands; ? Rutaceae |
| Unknown sp 19 | *Stoebe plumosa* – like |
| Unknown sp 20 | Herb |
| Unknown sp 21 | Herb |
| Unknown sp 22 | ? *Muraltia sp.*, ? *Metalasia sp.* |
| Unknown sp 23 | ? Herb, ? Tree seedling, *Myrsine africana* - like |
| Unknown sp 24 | Herb |
| Unknown sp 25 | Herb; *Phylica* – like |
| Unknown sp 26 | Defoliate bush |
| Unknown sp 27 | Herb |

**Appendix 3 – List of bee species**

| Sp. no. | Species | Species is known from which gender | Possible male partner of other female congener? |
|---|---|---|---|
| 1 | X*ylocopa rufitarsis* Lepeletier | Both | n/a |
| 2 | *Xylocopa caffra* (Linnaeus) | Female | n/a |
| 3 | *Xylocopa albifrons* Lepeletier | Female | n/a |
| 4 | *Apis mellifera* Linnaeus | Worker | n/a |
| 5 | *Tetraloniella nubilis*(Cockerell) | Male | n/a |
| 6 | *Anthophora wartmanni* Friese | Female | n/a |
| 7 | *Amegilla spilostoma* (Cameron) | Female | n/a |
| 8 | *Ceratina braunsi* Eardley & Daly | both | n/a |
| 9 | *Ceratina nyassensis* Strand | both | n/a |
| 10 | *Ceratina aliceae* Cockerell | female | n/a |
| 11 | *Allodape quadrilineata* (Cameron) | female | n/a |
| 12 | *Allodape panurgoides* Smith | female | n/a |
| 13 | *Megachile frontalis* Smith | female | n/a |
| 14 | *Spinanthidium callescens* (Cockerell) | female | n/a |
| 15 | *Serapista rufipes* (Friese) | male | n/a |
| 16 | *Coelioxys (Liothyrapis)* sp. | female | n/a |
| 17 | *Heriades* sp. 1 | female | n/a |
| 18 | *Heriades* sp. 2 | female | n/a |
| 19 | *Heriades* sp. 3 | female | n/a |
| 20 | *Heriades* sp. 4 | male | Possible |
| 21 | *Heriades* sp. 5 | male | Possible |
| 22 | *Haetosmia* sp. 1 | female | n/a |
| 23 | *Haetosmia* sp. 2 | female | n/a |
| 24 | *Colletes* sp. 1 | female | n/a |
| 25 | *Colletes* sp. 2 | female | n/a |
| 26 | *Hylaeus* sp. 1 | female | n/a |
| 27 | *Hylaeus* sp. 2 | female | n/a |
| 28 | *Scrapter opacus* (Cockerell) | female | n/a |
| 29 | *Scrapter nitidus* (Friese) | both | n/a |
| 30 | *Scrapter erubescens* (Friese) | female | n/a |
| 31 | *Scrapter* sp. | male | n/a |
| 32 | *Lasioglossum* sp. 1 | female | n/a |
| 33 | *Lasioglossum* sp. 2 | female | n/a |
| 34 | *Lasioglossum* sp. 4 | female | n/a |
| 35 | *Lasioglossum* sp. 5 | female | n/a |
| 36 | *Lasioglossum* sp. 6 | female | n/a |

**Appendix 3 – continued**

| Sp. | Species | Species is | Possible male |
|---|---|---|---|

| no. | | known from which gender | partner of other female congener? |
|---|---|---|---|
| 37 | *Lasioglossum* sp. 7 | female | n/a |
| 38 | *Lasioglossum* sp. 8 | female | n/a |
| 39 | *Lasioglossum* sp. 9 | female | n/a |
| 40 | *Lasioglossum* sp. 10 | female | n/a |
| 41 | *Lasioglossum* sp. 11 | female | n/a |
| 42 | *Lasioglossum* sp. 12 | female | n/a |
| 43 | *Lasioglossum* sp. 13 | female | n/a |
| 44 | *Lasioglossum* sp. 14 | female | n/a |
| 45 | *Lasioglossum* sp. 15 | female | n/a |
| 46 | *Lasioglossum* sp. 16 | female | n/a |
| 47 | *Lasioglossum* sp. 17 | female | n/a |
| 48 | *Lasioglossum* sp. 18 | female | n/a |
| 49 | *Pseudapis* sp. | male | n/a |
| 50 | *Halictus (Seladonia)* sp. 1 | female | n/a |
| 51 | *Halictus (Seladonia)* sp. 2 | female | n/a |
| 52 | *Nomioides* sp. 1 | female | n/a |
| 53 | *Nomioides* sp. 2 | male | Possible |
| 54 | *Ceylalictus* sp. | female | n/a |
| 55 | *Patellapis* sp. 1 | female | n/a |
| 56 | *Patellapis* sp. 2 | female | n/a |
| 57 | *Patellapis* sp.3 | female | n/a |
| 58 | *Patellapis* sp. 4 | female | n/a |
| 59 | *Patellapis* sp. 5 | female | n/a |
| 60 | *Patellapis* sp. 6 | female | n/a |
| 61 | *Patellapis* sp. 7 | male | Possible |
| 62 | *Patellapis* sp. 8 | male | Possible |
| 63 | *Patellapis* sp. 9 | male | Possible |
| 64 | *Patellapis* sp. 10 | male | Possible |
| 65 | Unknown 1 *A. mellifera* – like | unknown | n/a |
| 66 | Unknown 2 h" | unknown | n/a |

**Appendix 4 - Pictures of research sites.**

Sites 1 and 2 are from a Google Earth picture in order to encompass both sites. (i) Sites 1 and 2. Site 1 (above) is at a higher elevation. (ii) Site 3. (iii) Site 4. (iv) Site 5.

(i)

(ii)

Appendix 4. (continued) (iii) Site 4. (iv) Site 5.

(iii)

(iv)